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Original Article

Assessment of Plasma Iron, Feritin, Blood Platelets and Hemoglobin Concentration in Rabbits Given Amoxicillin Overdose Supplemented with Raw Cucumber Juice

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ABSTRACT

Background and Aim: Raw Cucumber (*Cucumis sativus*) fruit juice contains substances of health promoting bioactivities. Plasma iron is stored in the liver and blood cells. Platelets are blood cells involved in blood clotting for homeostasis while haemoglobin (found on red blood cells) concentration is an index of anaemia and polycythemia. This work was therefore designed to assess plasma iron, feritin, blood platelets and hemoglobin concentration in rabbits given amoxicillin overdose supplemented with raw cucumber fruit Juice. **Methods:** Fifteen rabbits of the same sex weighing 0.9 – 1.4Kg divided into 3 groups of 5 rabbits each were used for the study. Group A- 5 control rabbits; Group B –5 rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days which was followed by 30ml raw cucumber fruit juice supplementation for 14 days; Group C–5 rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs and raw cucumber fruit juice supplementation for 14 days simultaneously. Plasma iron, ferritin and hemoglobin concentration were determined in the rabbits biochemically by spectrophotometry and immunoturbidometry while platelets were enumerated by haemocytometry. **Results:** There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets when the rabbits were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days compared with the results obtained from the control rabbits, their basal samples and when they were supplemented with30ml raw cucumber fruit juice for 14 days with $p<0.05$. There was a significant decrease in plasma ferritin and iron with a significant increase in haemoglobin concentration and blood platelets when they were supplemented with3 0ml raw cucumber fruit juice for 14 days compared with the results obtained when they were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days with $p<0.05$.Conclusion : There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets upon administration of amoxicillin overdose which was reversed when the supplement of raw cucumber fruit juice was given. Cucumber fruit juice possibly has a protective effect on blood cells and liver as there was no significant haematological alteration when amoxicillin overdose and raw cucumber fruit juice were co-administered.

Key words: Plasma iron, Ferritin, Blood platelets, Hemoglobin concentration, Amoxicillin overdose, Raw cucumber juice.

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INTRODUCTION

Cucumber (*Cucumis sativus*) juice is produced from cucumber fruit by squeezing or pressing the blended of sliced fruit on a sterile sieve. Cucumbers contains 98% water, phytonutrients vitamin A, vitamin C, vitamin K, calcium,, potassium, silicon, copper, calcium), large amounts of sterol, both soluble and insoluble fiber.[1-4] It has antioxidant and cholesterol lowering properties.[5-7] It is used in traditional medicine to treat inflammation of respiratory tract, toxicity, cough, heartburn, stomach burns and rashes.[6-7]Amoxicillin is a β-lactam antibiotics used for the treatment of bacterial infections such as middle ear infections, strep throat, pneumonia, skin infections, enteritis and urinary tract infections.[8] The side effects include: nausea, vomiting, rashes, antibiotic-associated colitis, loose bowel movements (diarrhea), mental changes, lightheadedness, insomnia, confusion, anxiety, sensitivity to lights and sounds and unclear thinking, while its overdoses can cause lethargy, bleeding, hepatitis, vomiting and renal dysfunction.[9-10] Iron is a mineral required by the body for many functions such as being a part of hemoglobin on the red blood cells. It helps body muscles store and use oxygen. Iron is also a part of many other proteins and enzymes. It is stored in the liver and also essential for haemopoesis.[11,12] Ferritin is an intracellular and an acute phase protein that stores iron and releases iron to maintain balance. Ferritin is produced by almost all living organisms, (algae, bacteria, higher plants and animals). It function as

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a buffer in iron deficiency and iron overload. Increased plasma ferritin (an acute phase protein) could be in response to stresses such as anoxia.[13,14] Plasma ferritin is also an indirect marker of the total amount of iron stored in the body, It can be used as a diagnostic test for iron-deficiency anemia.[15,16] Ferritin stores iron in a non-toxic form, to be able to deposit it in a safe form and also for transportation to areas where it is needed. The function and structure of ferritin varies with cell types. Ferritin concentrations increase in infection or cancer.[13-16] Haemoglobin is the iron-containing oxygen-transport protein that constitute about 96% of the red blood cells' dry content and around 35% of the total content including water. It carries oxygen from the respiratory organs such as lungs, gills to tissues and cells. Haemoglobin releases the oxygen for aerobic respiration and metabolism. It has an oxygen-binding capacity of 1.34 mL O_2 per gram.[17,18] Hemoglobin is a marker of anaemia and also transports body's respiratory carbon dioxide (about 20–25% of the total as carbaminohemoglobin, in which CO_2 is bound to the globin protein.[19,20] Platelets are blood cells that aggregate when blood vessels are injured to stop bleeding. They circulate in the body through blood and responsible for blood clotting.[21,22] Cucumber is a common fruit used as a beverage and applied in traditional treatments. Amoxicillin is one of the commonest antibiotic used to treat infections such as typhoid enteritis, respiratory and urinary tract infection. It is easily accessible and as such could be abused. There is little information on supplementation of amoxicillin overdose or toxicity with cucumber juice with respect to plasma iron, ferritin, blood platelets and hemoglobin concentration hence the need for this work. This work was to assess the plasma iron, feritin, haematocrit and hemoglobin concentration in rabbits given amoxicillin overdose and supplemented with raw cucumber juice.

MATERIALS AND METHODS

Materials

Study area

This work was carried out at the Animal house of Achievers University, Owo-Nigeria. Achievers University is in Owo, Ondo State Nigeria. The university is a private-sector initiative, established in 2007 and accredited by the National Universities Commission. It is located on land in the Idasen community of Owo, consisting of Ulale 1, Ulale 11, Ulema, Ijegunma, Isijogun and Amurin Elegba (formerly Amurin, Ogain). The university sprang from the Achievers Group of Education and Training Organization, located in Ibadan Oyo State of Nigeria owned and run by Hon. Dr Bode Ayorinde and other educationalists. The university commenced academic activities during the 2007/2008 academic session. In the Nigerian National University Commission annual university rankings for 2013, it was rated 53rd. It has three Colleges which include College of Natural and applied Sciences; College of Engineering and Technology and College of Social Science and Management including a Postgraduate school.

Study population

Fifteen rabbits of the same sex divided into 3 groups of 5 rabbits each was used for the study. The rabbits was bought animal farm in Owo-Nigeria and was presented to Federal School of Agriculture, Akure for confirmation.

Group A- 5 control rabbits were fed with normal meal and water throughout the period of investigation.

Group B– 5 rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days which was followed by 30ml raw cucumber fruit juice supplementation for 14 days.

Group C– 5 rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs and raw cucumber fruit juice supplementation for 14 days simultaneously

Administration of amoxicillin

Amoxicilin was bought from a registered pharmaceutical shop in Owo-Nigeria. Overdose of 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days.

Preparation of Cucumber (*Cucumis sativus*) fruit juice

Cucumber (*Cucumis sativus*) was bought from fruit vendors in Owo- Nigeria. The fruit was presented to Federal School of Agriculture, Akure for confirmation. The fruit was washed in sterile water and then sliced. The sliced fruit was blend together using an electronic blender. The raw fluid was extracted using a sterile sieve. The raw liquid extract was served to the rabbits as juice. 30ml was given to the rabbits on daily basis. The juice will always be freshly prepared.

Specimen (blood) collection

Five milliliters of venous blood was collected from each of the rabbits into EDTA and lithium heparinized bottles for haematological analysis.

Haematological assays

Plasma Iron using RANDOX Kit

Principle: Ferric iron is dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is then complexed with the chromogen, a sensitive iron indicator, to produce a blue chromophore which absorbs maximally at 595 nm**.**

Plasma ferritin

Principle: When an antigen-antibody reaction occurs between ferritin in a sample and an anti-ferritin antibody, which has been sensitized to latex particles, agglutination occurs. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of ferritin in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known ferritin concentration.

Hemoglobin concentration assay using RANDOX Kit

Principle: Hemoglobin is first oxidized by potassium ferricyanide into methemoglobin which is converted into cyanmethemoglobin by potassium cyanide. The absorbance of the cyanmethemoglobin is monitored at 540 nm.

Platelet count was carried out as described by Cheebrough[23]Principle: Whole blood is diluted with a 1% ammonium oxalate solution. The isotonic balance of the diluent is such that all erythrocytes are lysed while the leukocytes, platelets and reticulocytes remain intact. The standard dilution for platelet counts is 1:100. This dilution is prepared using the leukocyte/platelet Unopette system.1 The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on a hemacytometer. The cells are allowed to settle and then are counted in a specific area of the hemacytometer chamber under the microscope. The number of platelets is calculated per µL (x 109 /L) of blood.

Statistical analysis of data

The information from this study was subjected to statistical analysis to determine mean, standard deviation, students 't' and probability value at 0.05 level of significance using SPSS 18.0.

RESULTS

There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets when

the rabbits were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days compared with the results obtained from the control rabbits, their basal samples and when they were supplemented with 30ml raw cucumber fruit juice for 14 days with $p<0.05$ (Table1, 2; Figure 1).

There was a significant decrease in plasma ferritin and iron with a significant increase in haemoglobin concentration and blood platelets when they were supplemented with 30ml raw cucumber fruit juice for 14 days compared with the results obtained when they were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days with $p<0.05$ (Table 1, 2; Figure 1).

There was no significant difference in the value of plasma ferritin, iron, haemoglobin concentration and blood platelets obtained from the rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs and raw cucumber fruit juice supplementation for 14 days simultaneously $p>0.05$ (Table1,2; Figure 1).

There was no significant difference in the value of plasma ferritin, iron, haemoglobin concentration and blood platelets obtained from the rabbits' basal samples, control rabbits and the results obtained after 30ml raw cucumber fruit juice supplementation for 14 days $p>0.05$ (Table1, 2; Figure 1).

DISCUSSION

There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets when the rabbits were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days compared with the results obtained from the control rabbits, their basal samples and when they were supplemented with 30ml raw cucumber fruit juice for 14 days. These findings could be as a result of destruction of red blood cells, platelets and liver damage possibly caused by amoxicillin overdose.[9,10] As ferritin and iron are found in the liver and red blood cells. The substances are release into the circulation upon red blood cell hemolysis and liver cell damage/hepatotoxicity haemopoesis.[11,12] Furthermore, ferritin levels may be artificially high in cases of anemia of chronic disease where ferritin is elevated in its capacity as an inflammatory acute phase protein and not as a marker for iron overload. As ferritin is also an acute-phase reactant, it is often elevated in the course of disease. Furthermore, ferritin is a positive acute phase protein and also has significant non storage roles within the body, such as protection from oxidative damage which could have been induced by amoxicillin over dose.[16,24] Decrease in haemoglobin concentration and blood platelets could be as a result of blood cells destruction by amoxicillin overdose and possible suppression of blood cells formation due to drug toxicity. In addition amoxicillin induced hepatotoxicity may reduce the responsibilities of the liver as it affects production of platelets because liver is a major site of production for thrombopoietin, a glycoprotein hormone that regulates the production of platelets by the bone marrow.[25]There was a significant decrease in plasma ferritin and iron with a significant increase in haemoglobin concentration and blood platelets when they were supplemented with 30ml raw cucumber fruit juice for 14 days compared with the results obtained when they were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days These findings could be attributed to health benefit bioactivities of raw cucumber fruit juice as Cucumber (*Cucumis sativus*) is a popular fruit crop very high in water content and very low in calories. It has potential antidiabetic, lipid lowering and antioxidant activity. It also contains vitamin C and K necessary for production of blood cells. Cucumber has a cleansing action within the body by removing accumulated pockets of old waste materials and chemical toxins.[26] Cucumber juice contains large amounts of vitamin A, vitamin C, vitamin K, calcium, potassium, silicon, copper, calcium required for haemoglobin and blood cells production.[1-7] There was no significant difference in the value of plasma ferritin, iron, haemoglobin concentration and blood platelets obtained from the rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs and raw cucumber fruit juice supplementation for 14

Table 1: Mean and Standard Deviation of Plasma Iron /µg/dL Ferritin/ µg/L, Hb (g/dl) and Blood platelets/109/L obtained in the rabbits.

	Control rabbits A	Group B			Group C	
		B1	B2	B3	C1	C2
Plasma Iron /µg/dL	28±1.0	30 ± 2.0	48 ± 3.0	34 ± 5.0	26 ± 3.0	30 ± 1.0
Ferritin/ µg/L	11± 1.0	10 ± 2.0	19 ± 1.0	11 ± 1.0	12 ± 2.0	11 ±2.0
Hb (g/dl)	8.0 ± 0.3	8.1 ± 0.2	5.4 ± 0.4	7.9 ± 0.1	8.1 ± 0.1	8.0 ± 0.2
Blood platelets/109/L	142 ± 10.0	145 ± 10.0	101 ± 10.0	140 ±9.0	141 ± 10.0	143 ± 10.0

B1- basal blood samples; B2- blood samples after amoxicillin injection; B3- blood samples after administration of cucumber juice following amoxicillin injection; **C1**- basal blood samples; **C2**- blood samples after simultaneous administration of cucumber juice and amoxicillin injection.

Table 2: Comparative analysis of the Mean and Standard Deviation of Plasma Iron /µg/dL Ferritin/ µg/L, Hb (g/dl) and Blood platelets/109/Lobtained in the rabbits

		A vs B1	Avs B2	Avs B3	Avs C1	Avs C2	B1 vs B2	B1vs B3	B2vs B3	C1 vs C2
Plasma Iron / µg/dL	't'	-0.894	-6.325	-1.177	0.633	-1.414	-4.99.	-0.74	2.40	-1.27
	'*p*'	0.23	0 .012*	0 .181	0 .296	0.147	0.017*	0 .268	0.069	0 .17
Ferritin/ µg/L	't'	0.447	-5.66	0.	-0.45	0.	-4.025	-0.447	5.66	0.354
	'*p*'	0.35	0 .015*	0 .5	0 .349	0 .5	0.028*	0.349	0.02*	0 .379
Hb (g/dl)	't'	-0.277	5.2.	0.316	-0.316	0.	6.037	0.894	-6.063	0.447
	'*p*'	0 .404	0 .018*	0 .39	0 .391	0 .5	0 .01*	0 .233	0.013*	0 .349
Blood platelets/109/L	't'	-0.212	3.173	0.149	0.071	-0.071	3.43	0.37	-3.24	-0.141
	'*p*'	0.426	0 .043*	0 .448	0 .475	0.475	0.038*	0 .37	0.042*	0 .4502

*Significant

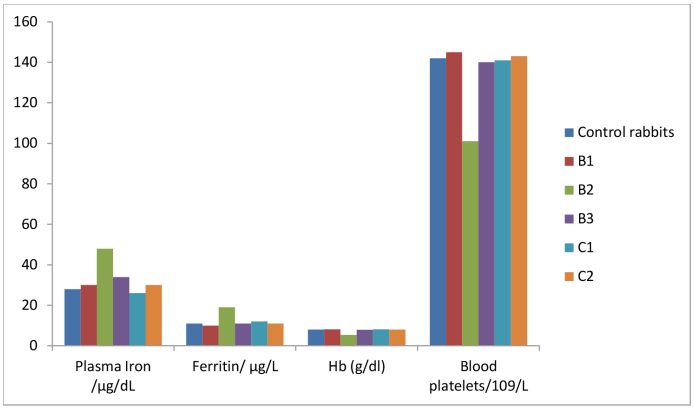

Figure 1: Comparative description of the Mean and Standard Deviation of Plasma Iron/µg/dL Ferritin/ µg/L, Hb (g/dl) and Blood platelets/109/L obtained in the rabbits

days simultaneously which could be attributed to its possible hepatoprotective effect due to antioxidant property of raw cucumber juice capable of preventing cellular damage.[1-7]

CONCLUSION

There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets upon administration of amoxicillin overdose which was reversed when the supplement of raw cucumber fruit juice was given. Cucumber fruit juice possibly has a protective effect on blood cells and liver as there was no significant haematological alteration when amoxicillin overdose and raw cucumber fruit juice were co-administered. Raw cucumber fruit juice could help in preventing organ/ cellular damage and possible natural treatment for low haemoglobin concentration.

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None

CONFLICT OF INTEREST

None

ABBREVIATIONS

None

SUMMARY

Raw Cucumber (*Cucumis sativus*) fruit juice contains substances of health promoting bioactivities. Plasma iron is stored in the liver and blood cells. Platelets are blood cells involved in blood clotting for homeostasis while haemoglobin (found on red blood cells) concentration is an index of anaemia and polycythemia. This work was therefore designed to assess plasma iron, feritin, blood platelets and hemoglobin concentration in rabbits given amoxicillin overdose supplemented with raw cucumber fruit Juice. Fifteen rabbits of the same sex weighing 0.9 – 1.4Kg divided into 3 groups of 5 rabbits each were used for the study. Group A- 5 control rabbits; Group B –5 rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days which was followed by 30ml raw cucumber fruit juice supplementation for 14 days; Group C – 5 rabbits given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs and raw cucumber fruit juice supplementation for 14 days simultaneously. Plasma iron, ferritin and hemoglobin concentration were determined in the rabbits biochemically by spectrophotometry and immunoturbidometry while platelets were enumerated by haemocytometry. There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets when the rabbits were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days compared with the results obtained from the control rabbits, their basal samples and when they were supplemented with30ml raw cucumber fruit juice for 14 days with $p<0.05$. There was a significant decrease in plasma ferritin and iron with a significant increase in haemoglobin concentration and blood platelets when they were supplemented with 30ml raw cucumber fruit juice for 14 days compared with the results obtained when they were given 30.0mg/kgBW subcutaneous injection of amoxicillin every 24 hrs for 7 days with $p<0.05$. There was a significant increase in plasma ferritin and iron with a significant decrease in haemoglobin concentration and blood platelets upon administration of amoxicillin overdose which was reversed when the supplement of raw cucumber fruit juice was given. Cucumber fruit juice possibly has a protective effect on blood cells and liver as there was no significant haematological alteration when amoxicillin overdose and raw cucumber fruit juice were co-administered.

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