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Studies of cow urine and its effect on hematological and histopathological analysis of pathogen-induced animal

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Abstract

The effect of fresh, photo-activated and distilled cow urine was examined on the hematological parameters of pathogen-induced albino rats using automated hematological analyzer. The albino rats were grouped into 3 (A, B and C) and 2 controls; with each group containing 5 rats. Each animal group was induced with 0.5mL of *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* respectively. After 7 days of infection, each animal group was treated with fresh, photo-activated and distilled cow urine for the period of 5 days. The animal group infected with *P. aeruginosa* died after second day of treatment. The blood samples of the animal were collected for hematological analysis. The parameters taken are; Packed Cell Volume (PCV), Hemoglobin (Hb), White Blood Cell count (WBC), Neutrophil, Lymphocytes, Monocytes, Eosinophil and Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell count (RBC). The result obtained from the analysis showed that all the samples of cow urine used were effective in the treatment of diseases caused by the bacterial pathogens.

Keywords: Assessment; Cow urine; Hematological; Pathogen-Induced

1. Introduction

Cow *(Bos indicus)* urine/*gomutra* has been elaborately explained in Ayurveda and described in *"Sushruta Samhita", "Ashtanga Sangraha"* and other Ayurvedic texts as an effective medicinal substance/secretion of animal origin with innumerable therapeutic properties [1]. Bhav Prakash Nighantu describes *gomutra* as the best of all types of animal urine (including human) and enumerates its various therapeutic uses [2]. Persons who drink *gomutra* regularly are said to live a healthy life, remaining unaffected by the vagaries of old age, even at age 90 [3]. *Gomutra* is called *"Sanjivani"* and "*Amrita*" in Ayurveda. In addition, it has applications as a biopesticide in organic farming along with cow dung, cow's milk and other herbal ingredients [4].

Gomutra is not a toxic waste material. 95% of it is water, 2.5% consists of urea, and the remaining 2.5% is a mixture of minerals, salts, hormones and enzymes [5]. *Gomutra* exhibits the property of *Rasayana tattwa* responsible for modulating various bodily functions, including immunity. It augments B- and T-lymphocyte blastogenesis; and IgG, IgA and IgM antibody titers in mice. It also increases secretion of interleukin-1 and interleukin-2 [6], phagocytic activity of macrophages, and is thus helpful in the prevention and control of infections.

Antimicrobial and germicidal properties of *gomutra* are due to the presence of urea (strong effect), creatinine, swarn kshar (aurum hydroxide), carbolic acid, other phenols, calcium and manganese; its anticancer effect is due to uric acid's antioxidant property and allantoin; immunity is improved by swarn kshar; and wound healing is promoted by allantoin. Cardiovascular health is maintained by a number of its components: kallikrein is a vasodilator; the enzyme urokinase

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acts as a fibrinolytic agent; nitrogen, uric acid, phosphates and hippuric acid act as diuretic agents; ammonia maintains the integrity of blood corpuscles; nitrogen, sulfur, sodium and calcium components act as blood purifiers; while iron and erythropoietin stimulating factor maintain hemoglobin levels. Renal health is maintained by nitrogen, which acts as a renal stimulant, and urinary components which act as diuretic agents. Its antiobesity effect is due to the presence of copper ions; calcium promotes skeletal/bone health. Aurum hydroxide and copper act as antidotes for various poisons in the body [7].

Cow urine exhibits antitoxic activity against cadmium chloride and can be used as a bioenhancer for zinc, Zn^{2+}. Mature male mice, *Mus musculus*, exposed to cadmium chloride only, showed 0% fertility rate. However, the animals given a combination of cadmium chloride + cow urine + zinc sulfate showed 90% fertility rate with 100% viability and lactation indices. Besides this, the fertility index was also found to be 88% in the group treated with cadmium chloride and cow urine [6].

There are so many claims regarding the use of cow urine. Out of these the most important claim is regarding its antidiabetic and antioxidant activity, but only few scientific literatures are available to support this claim [8].

Hematological parameters such as red (RBC) and white blood cell (WBC) counts and hemoglobin (Hb) concentration are tightly regulated traits with high clinical relevance. Values outside normal ranges are diagnostic for disorders, including cancer, immune diseases, and cardiovascular disease. In humans, hematological parameters have heritabilities >50% [9], and mutations in key genes have important phenotypic consequences. Hemoglobinopathies, including the hemoglobin β S allele that plays a role in malarial resistance, are widespread in human populations and are among the most intensively studied human genetic diseases [10]. The objective of this project study is to examine the effect of fresh, photo-activated and distilled cow urine on hematological parameters of pathogen-induced animals

2. Material and methods

2.1. Experimental Animals

Three to four weeks old, eighteen (18) albino rats (*Rattus norvegicus*) of both sexes weighing 170 - 190 g were obtained from the small Animal House of the Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Nigeria. The animals were housed in aluminum cages placed in well-ventilated house with optimum conditions with free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water.

2.2. Collection and preparation of cow urine

Fresh cow urine was collected from Abattoir located along Iworoko Road, Ado-Ekiti. The samples were collected into sterile sample bottle. It was brought to the laboratory and filtered. Photo-activated cow urine was prepared by keeping fresh cow urine in sunlight for 72 hours in sealed glass bottle. The sample was then filtered through Whatman No. 1 filter paper to get rid of debris and precipitated material was stored at 4 °C before use. Before evaluation of antibacterial activity, the sample was checked for the presence of microbial contamination. Cow urine distillate was obtained by distilling cow urine at 100 °C using distillation apparatus.

2.3. Collection of test organisms

The test organisms used in the study were *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*. The entire test cultures were obtained from the Microbiology laboratory. The purity of all the culture was checked before use. The cultures were maintained at 4 °C on nutrient agar slants.

2.4. Animal bioassay

2.4.1. Grouping and induction of animal samples with pathogens

Albino rat samples were grouped into 3 (A, B and C) with 2 controls. Each of the group contains 5 animal samples; each was injected with 0.5mL of the pathogens (*P. aeruginosa* , *E. coli* and *S. typhi*) for two days. After the injection the animal samples were observed for the period of 7 days for physical changes. The stool samples of the experimental animal were collected for confirmation of the presence of the organisms; and this was confirmed serial dilution the stool samples on prepared nutrient agar plates and incubated at 37 °C for 24 hours.

2.4.2. Administration of cow urine and collection of blood

After the observation, 0.5mL of fresh, photo-activated and distilled cow urine was administered to the animal samples of group A, B and C respectively on daily basis for five days. Group A animal which was injected with *P. aeruginosa* died the second day after treatment with fresh cow urine. Group B and C animal samples were sacrificed after the last dose and thereafter, hematological parameters were evaluated in the experimental animals.

2.4.3. Blood collection

Blood samples for hematological analysis were collected from the tip of the rats. The rats were anaesthetized in a jar containing cotton wool soaked in chloroform, they were then sacrificed by jugular puncture and their blood collected into an unheparinized bottle and allowed to stand for 10 minutes to clot. Serum was then collected using a Pasteur pipette.

2.4.4. Hematology

The blood samples were taken to hematological laboratory and diagnosis on the following parameters were taken; Packed Cell Volume (PCV), Hemoglobin (Hb), White Blood Cell count (WBC), Neutrophil, Lymphocytes, Monocytes, Eosinophil, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell count (RBC) using automated hematological analyzer.

3. Results

The results obtained from this study shows the effect of cow urine on the hematological parameters carried out on blood samples collected from the experimental animals infected with pathogens (*E. coli* and *S. typhi*) after treatment. The effect of photo-activated and distilled cow urine was observed on Packed Cell Volume (PCV), Hemoglobin (Hb), White Blood Cell count (WBC), Neutrophil, Lymphocytes, Monocytes, Eosinophil, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell count (RBC) of the experimental animal.

The PCV of the animal group injected with *E. coli* and treated with photo-activated cow urine was 165% while the group treated with distilled cow urine was 172%. *S. typhi* was recorded to be 52% and 84% for photo-activated and distilled cow urine respectively. The level of Hemoglobin (Hb) was recorded at 56 g/dL and 65 g/dL on *E. coli* animal group treated with photo-activated and distilled cow urine respectively; while the Hb levels of *S. typhi* animal group treated with photo-activated and distilled cow urine was recorded to be 17.3 g/dL and 28.0 g/dL respectively. White blood cell count (WBC) was observed to be 14.5 x 10^9/L and 17.2 x 10^9/L on *E. coli* animal group treated with photo-activated and distilled cow urine respectively; while the WBC of *S. typhi* animal group treated with photo-activated and distilled cow urine was recorded to be 15.0 x 10^9/L and 18.7 x 10^9/L respectively. Lymphocytes of the animal group injected with *E. coli* and treated with photo-activated cow urine was 73% while the group treated with distilled cow urine was 75%. *S. typhi* was recorded to be 71% and 72% for photo-activated and distilled cow urine respectively. Another interesting parameter was the RBC which was observed to be 21.6 x 10^{12}/L and 23.7 x 10^{12}/L *E. coli* animal group treated with photo-activated and distilled cow urine respectively; while the *S. typhi* animal group treated with photo-activated and distilled cow urine was observed to be 8.5 x 10^{12}/L and 14.2 x 10^{12}/L respectively.

Figure A1.1 is the section of liver showing nucleated cells and there was no inflammatory cell. Figure A1.2 is the section of the heart showing no anatomical changes of the cells and it is essentially normal. The kidney of the animal had normal glomeruli and the vessel appear normal as seen in figure A1.3 (Figure A1.1 – A1.3 are the sections of the organs of animal inoculated with *Staphylococcus aureus* and treated with antibiotics). Figure A2.1 is the section of liver showing normal cells and had no inflammatory cells. A2.2 is the section of the kidney which had its glomeruli and vessel normal (Figure A1.1 – A1.3 are the sections of the organs of animal inoculated with *Pseudomonas aeruginosa* and treated with antibiotics).

In figure B1.1, the section of the heart was normal; there was no anatomical change in the cells as well as no inflammatory cell. The section of the kidney (fig B1.2) showed inflammatory cells in the interstitial which was indicated with the arrow. Figure B1.3 is the section of the liver which had prominent kuffer cells and dilation of the sinusoids. The liver showed presence of neutrophils and lymphocytes in the central vein (Figure B1.1 – B1.3 are the sections of the organs of animal inoculated *Pseudomonas aeruginosa* with and treated with fresh obere cow urine). Figure B2.1 showed section of the liver of animal infected with *Staphylococcus aureus* which had hyperplasia, central vein and portal tracts appears free. Figure B2.2 showed the section of the kidney containing few inflammatory cells in the interstitial while the glomeruli appears normal. B2.3 is the section of the heart which was essentially normal.

Figure C1.1 showed section of heart which had nucleated cells with no inflammatory cells. C1.2 is the section of the liver with few inflammatory cells while C1.3 is section of the kidney which had some inflammatory cells but its glomeruli, vessels and interstitial appears normal (Figure C1.1 – C1.3 is the sections of the organs of animal inoculated *Pseudomonas aeruginosa* and treated with fresh nbala cow urine). In figure C2.1 (section of liver), there are generalized inflammatory cells while the kidney appeared normal as well as the heart (Figure C2.1 – 21.3 is the sections of the organs of animal inoculated with *Staphylococcus aureus* and treated with fresh nbala cow urine).

Table 1 Effect of cow urine on hematological parameters of experimental animal

Parameters	**Photo-activated cow urine**		**Distilled cow urine**	
	E. coli	***S. typhi***	***E. coli***	***S. typhi***
PCV	165%	52%	172%	84%
Hb	56 g/dL	17.3 g/dL	65 g/dL	28.0 g/dL
WBC	14.5 x 10^9 /L	15.0 x 10^9 /L	17.2 x 10^9 /L	18.7 x 10^9 /L
Neutrophil	8%	10%	9%	9%
Lymphocytes	73%	71%	75%	72%
Monocytes	73%	15%	17%	15%
Eosinophil	16%	4%	3%	4%
MCV	78 fL	62 fL	80 fL	59 fL
MCH	2.5 Pg	4.7 Pg	2.2 pg	4.0 pg
MCHC	3.2 g/dL	7.6 g/dL	2.8 g/dL	6.8 g/dL
RBC	21.6 x 10^{12} /L	8.5 x 10^{12} /L	23.7 x 10^{12} /L	14.2 x 10^{12} /L

g/dL– gram per deciliter; fL–Femtoliter ; Pg–Picogram; %–Percentage; /L –per liter

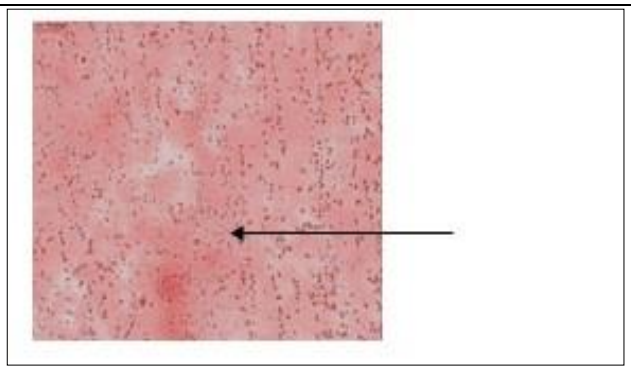


Figure A1.1 Normal cells of the liver

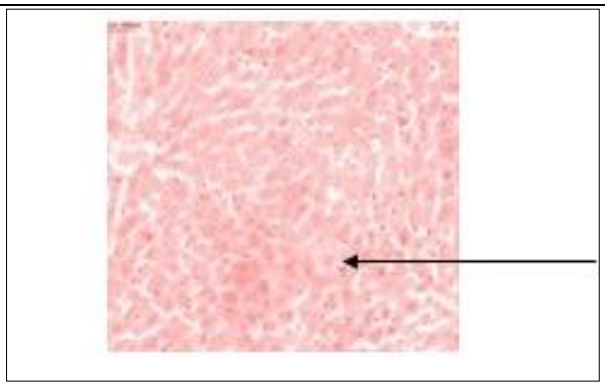


Figure A1.2 Section of the heart

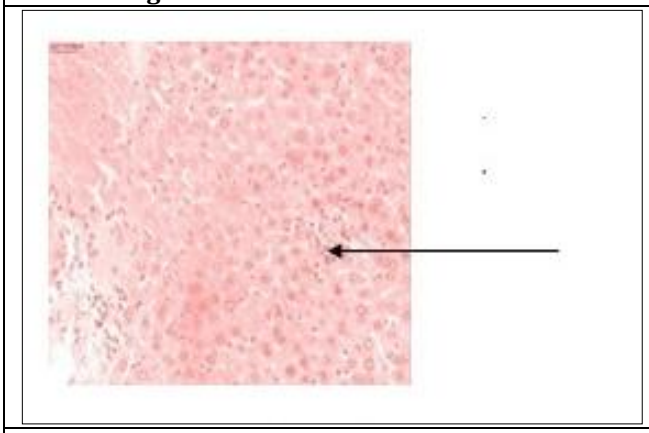


Figure A1.3 Section of kidney

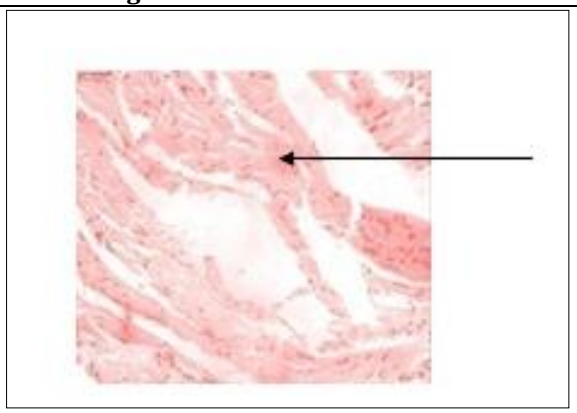


Figure A2.1 Section of heart

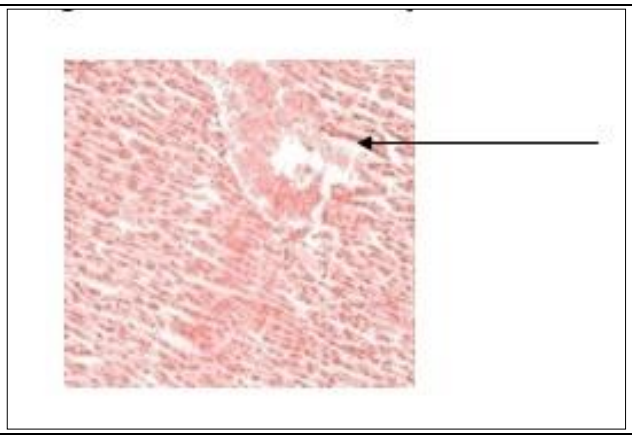


Figure A2.2 Section of heart

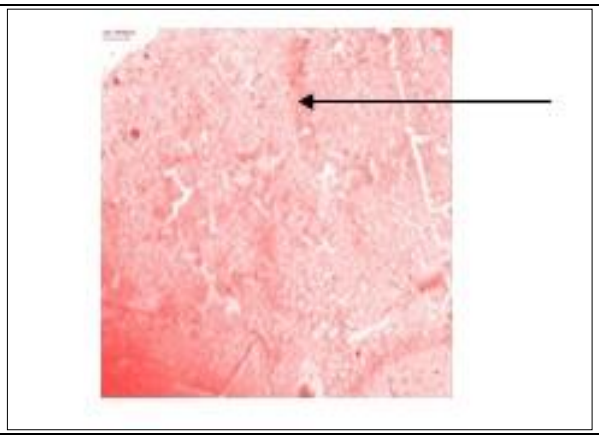


Figure A2.3 Section of kidney

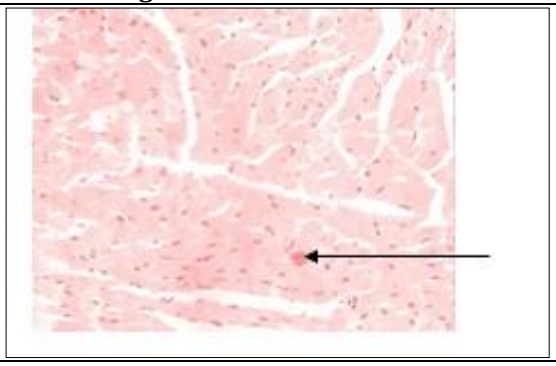


Figure B1.1 Section of heart

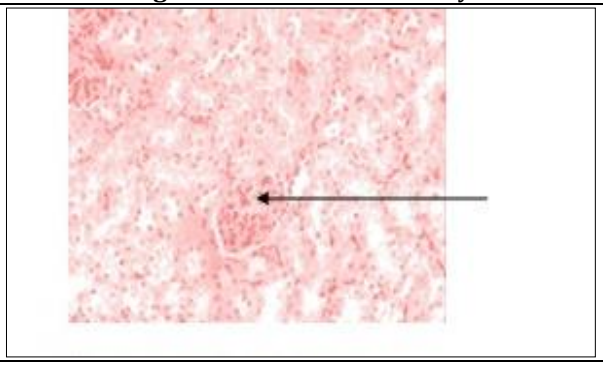


Figure B1.2 Section of kidney

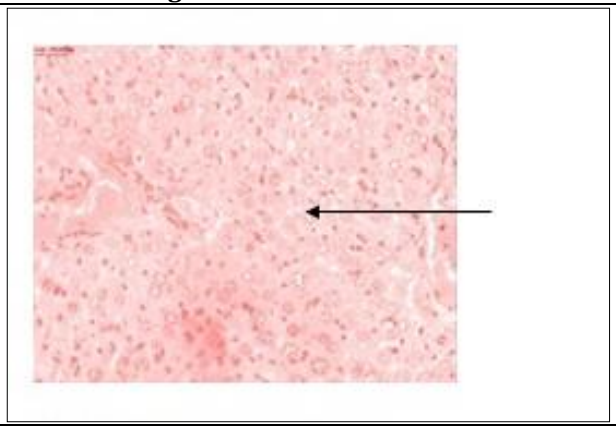


Figure B1.3 Section of liver

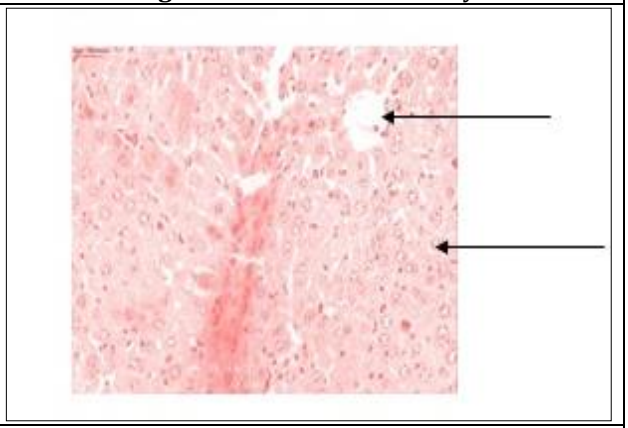


Figure B2.1 Section of liver

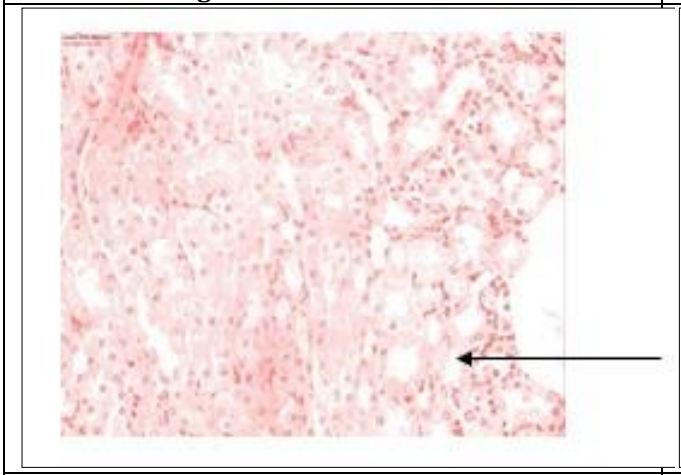


Figure B2.2 Section of kidney

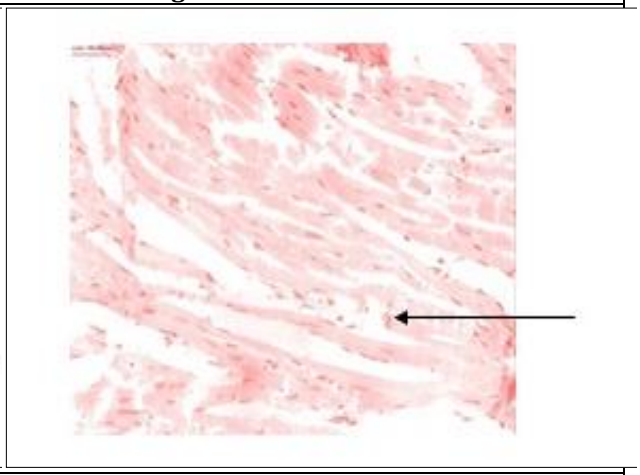


Figure B2.3 Section of heart

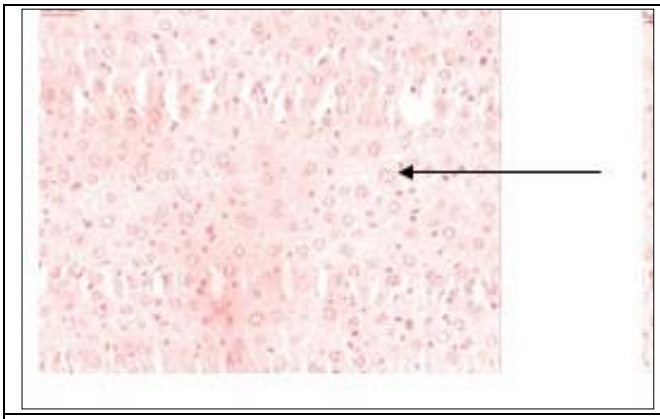


Figure C1.1 Section of heart

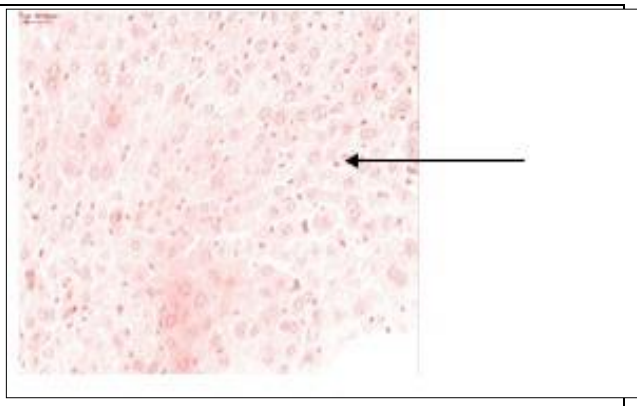


Figure C1.2 Section of liver

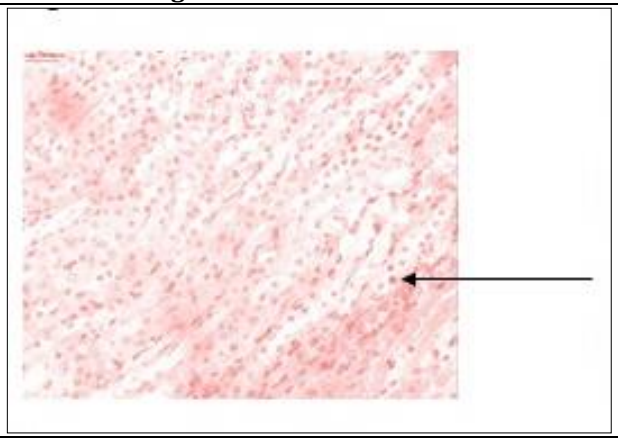


Figure C1.3 Section of kidney

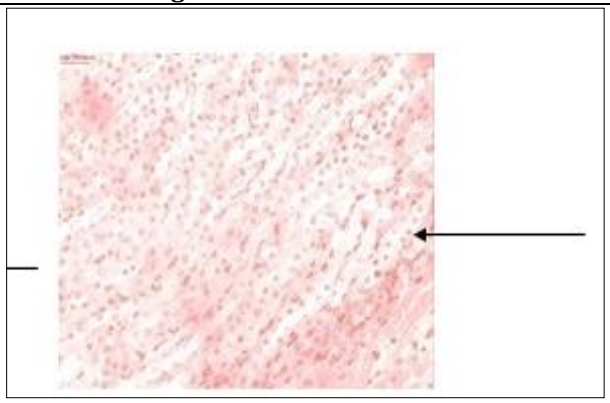


Figure C2.1 Section of liver

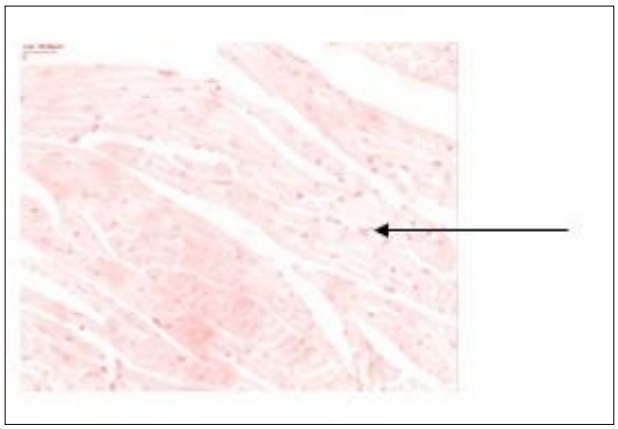


Figure C2.2 Section of heart

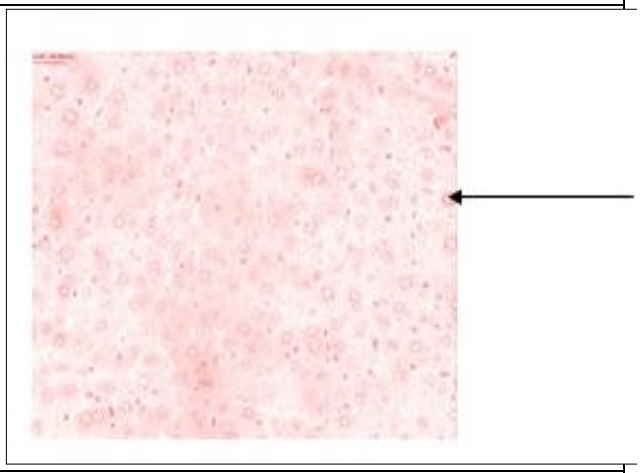


Figure C2.3 Section of kidney

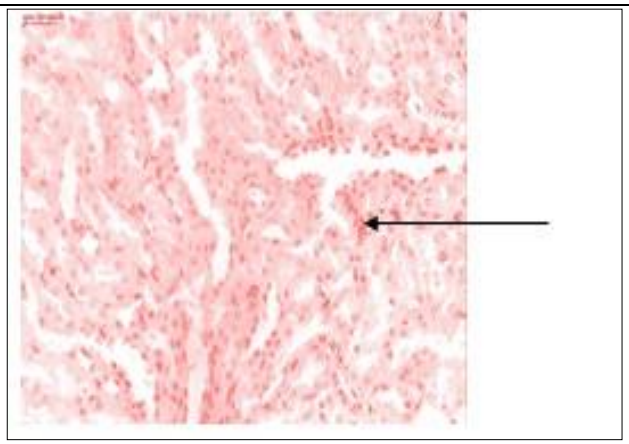


Figure D1.1 The heart

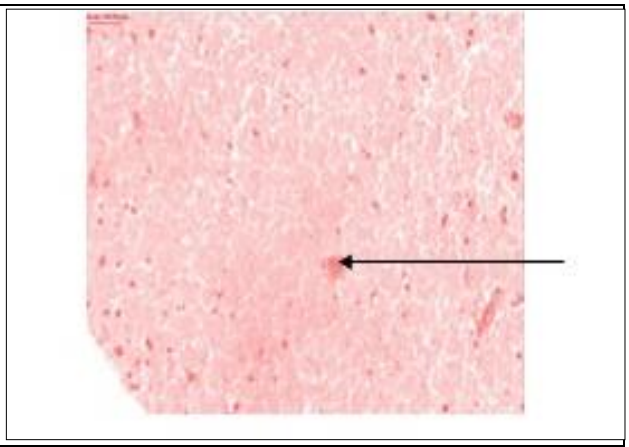
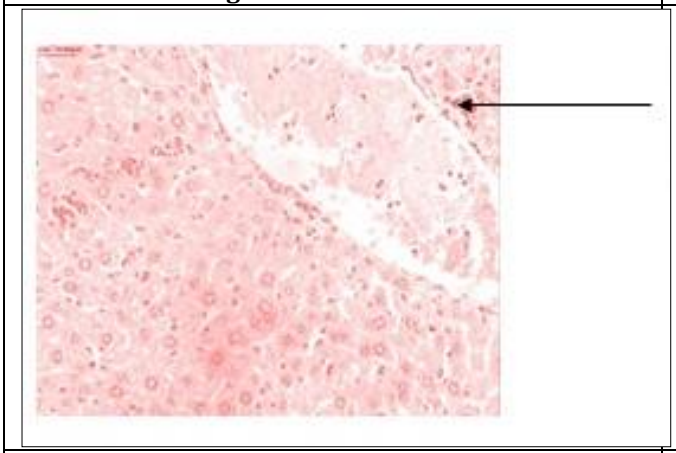


Figure D1.2 The liver



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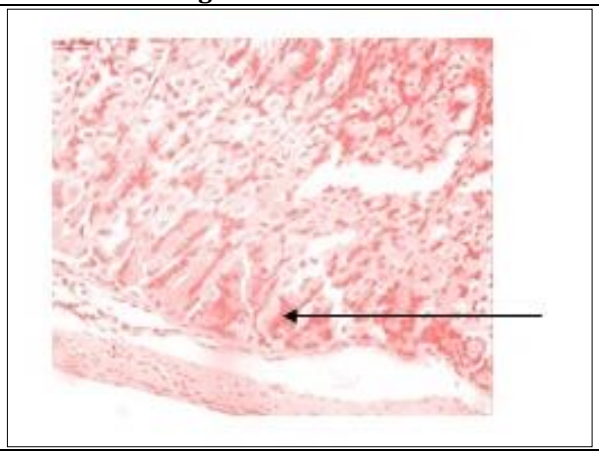

Figure D2.1 Section of heart

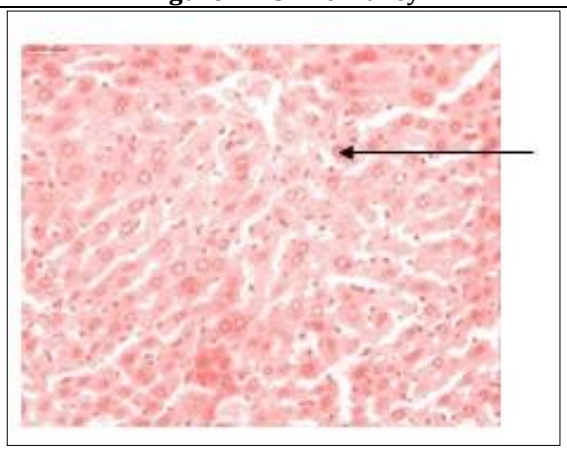

Figure D2.2 The kidney

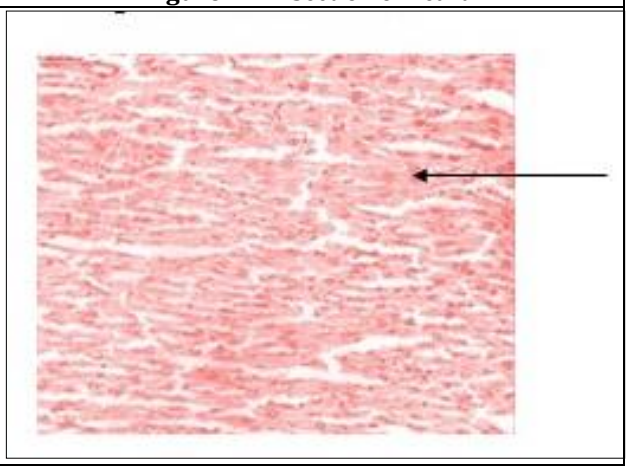

Figure D2.3 The liver

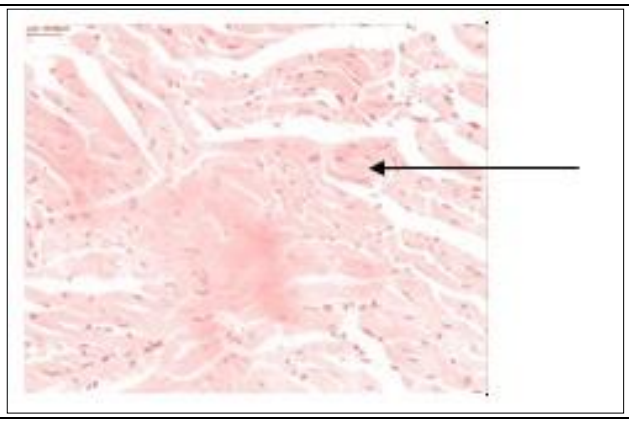

Figure E1.1 The heart

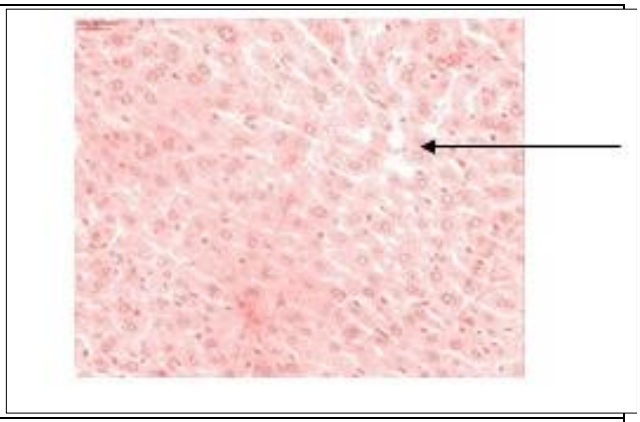

Figure E1.2 The liver

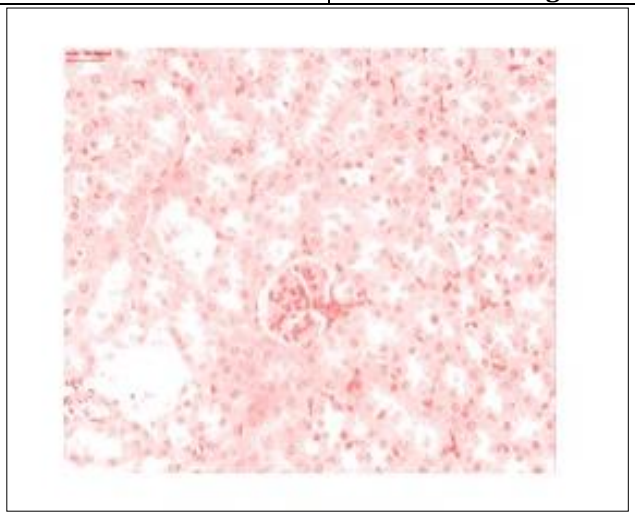

Figure E1.3 The kidney

In fig D1.1 the heart remains normal without inflammatory cells, fig1.2 showed the section of liver which had congestion of the bile duct and there are intense inflammatory cells. Fig D1.3 showed that the kidney contained some inflammatory cells in the glomeruli and the vessels appear normal. The heart (fig D2.1) had no infiltration and the organ remains normal. FigD2.2 which is the section of the kidney showed that there is no inflammatory cell and the liver fig D2.3 had scanty inflammatory cell.

The organs of group E (Fig E1.1, 1.2, 1.3) appear normal with no inflammation of the cell in heart, liver and kidney.

4. Discussion

The effect of photo-activated and distilled cow urine on hematological parameters were studied after 7 days of infection with bacterial pathogens and treatment on albino rats. The parameters like Packed Cell Volume (PCV), Hemoglobin (Hb), White Blood Cell count (WBC), Neutrophil, Lymphocytes, Monocytes, Eosinophil, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell count (RBC) were affected by cow urine samples. This corresponds with the study carried out by Verma *et al*. [11] who reported that hematological parameters like hemoglobin content, red blood cells count, white blood cell count and platelet count were affected by cow urine samples.

There were differences observed in efficacies of photo-activated and distilled cow urine in the percentages and levels of the hematological parameters analyzed on the animal group infected with *E. coli* and *S. typhi*. The potency of distilled cow urine treatment was higher than photo-activated cow urine on the animal group infected with *E. coli* owing to the differences in the percentages and levels of the hematological parameters. Except MCH and MCHC parameters where the values of photo-activated cow urine were higher than the distilled cow urine treatment; MCH of photo-activated cow urine treatment was 2.5pg while distilled cow urine was 2.2pg, and for MCHC the values were 3.2 g/dL and 2.8 g/dL respectively. There was no observed difference on Eosinophil parameter; both treatments had the values of 3%

respectively. This implies that photo-activated cow urine is more potent as far as these parameters (MCH and MCHC) are concerned.

The effect of distilled cow urine treatment on animal group infected with *S. typhi* was more pronounced compared to the photo-activated treatment in hematological parameters like PCV (84%), Hb (28.0 g/dL), WBC (18.7 x 10^9 /L), lymphocytes (72%) and RBC (14.2 x 10^{12} /L). But the photo-activated cow urine treatment was higher in hematological parameters like neutrophil (10%), MCV (62 fL), MCH (4.7pg) and MCHC (7.6 g/dL). The effect of both treatments was observed to be the same on monocytes (15%) and eosinophil (4%).

The histopathology of the organs showed that cow urine was not too toxic on the organs. The organs of the animals which were treated with antibiotics showed normal cells of the organs. All the cells were nucleated and there was no inflammation of the cell.

The organs of animals infected with *Pseudomonas aeruginosa* showed that the liver contained dilation of the sinusoids, prominent kuffer cells and there was necrosis of the cells which implies severe inflammation of the organ. The kidney of the animal showed normal glomeruli but there was presence of few inflammatory cells which implies mild tubulointerstitial nephritis. The heart had no anatomical changes. The organs of animals infected with *Staphylococcus aureus* and were treated with fresh cow urine of obere showed that the kidney of the animal contain mild inflammatory cells and the glomeruli appear normal and the implication of this is mild tubulointerstitial nephritis. The presence of kuffer cells in section of the liver and hyperplasia indicates mild necrosis. The heart showed that it is essentially normal. There is no indication of inflammatory cells.

The organs of animals treated with fresh nbala cow urine and infected with *Pseudomonas aeruginosa* had section of the heart normal without inflammation of the cells. Section of the liver had central vein and portal vein free and there was indication of few inflammatory cells that showed necrosis. In the section of liver of animal infected with *Staphylococcus aureus,* there was scanty inflammatory cells indicating mild necrosis, while the heart contain no inflammatory cells and is essentially normal, the kidney was also without inflammatory cells.

The heart of the animals treated with fermented urine and infected with *Pseudomonas aeruginosa* remain normal without inflammatory cells, while the section of liver had congestion of the bile duct and there are intense inflammatory cells which indicate severe necrosis. The kidney contained some inflammatory cells in the glomeruli and the vessels appear normal and this implies mild tubulointerstitial nephritis. The heart of the animals had no infiltration and the organ remains normal while the section of the kidney showed that there is no inflammatory cell and the liver had scanty inflammatory cell which indicates inflammation.

The organs of animals treated with fermented nbala appear normal with no inflammation of the cell in heart, liver and kidney.

From the discussion above, all the organs of the animals infected with *Pseudomonas* especially the liver were necrotic and this support the claim that gram –ve organisms are resistant to antibiotics while the organs of staphylococcus infected animals are moderately okay. Also the organs of the animals that were treated with fermented urine had mild toxicity.

Cow urine has been reported with beneficial properties for the cure diseases of human beings and thus regular use of cow urine may make the person free from many diseases [11].

5. Conclusion

The use of herbs and minerals for improving the overall resistance of body against common infections and pathogens has been a guiding principal of alternative medicine and therefore the composition of cow urine with its mineral content may be responsible for its effects on hematological parameters.

Recommendations

Photo-activated and distilled cow urine showed good effect on hematological parameters; hence it can be suggested that the photo-activated and distilled cow urine can improve the overall non-specific parameters of immunity. Therefore these fractions of cow urine are recommended for use. And also, further research should be carried out to determine its effect on biochemical parameters such as glucose level, cholesterol, albumin, etc.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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