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THE EFFECTS OF COLLOIDAL SILVER SOLUTION ON SOME HAEMATOLOGICAL PARAMETERS USING LABORATORY ANIMALS AS MODELS

Umeji LC[1*], Sanni FO[2], Umeji CN[3], Okolo CS[1], Okeke NC[1], Eigege NI[1], Ogundeji AA[4], Madukwe UJ [5], Ajobiewe OJ[5]

[1]Defence Reference Laboratory, Asokoro, Abuja, Nigeria,
[2]West African Breast Cancer Study Department, Lagos State University Teaching Hospital, Ikeja, Lagos State, Nigeria,
[3]Department of Chemical Pathology, Federal Medical Centre, Jabi, Abuja, Nigeria,
[4]United State Department of Defence, Walter Reed Program-Nigeria, US Embassy, Abuja, Nigeria,
[5]National Hospital Abuja, Plot 132 Garki Central District, Nigeria.

***Author for Correspondence:** st_armanie@yahoo.com

ABSTRACT

Exposure to a low dose of silver has been considered safe but recent studies have shown that long-time exposure to silver is harmful. The objective of this study is to evaluate the effects of colloidal silver solution on the haematological parameters of albino rabbits orally treated with 2.5ml per day of the solution for 3 months and 6 months. Twenty-two Albino rabbits were grouped into two of 11 Rabbits. Group one (subjects) was treated with Silver solution for six months and fed with commercially prepared rabbit pellets and clean water, while the group two (control) was fed with only commercially prepared rabbit pellets and clean water for six months. The drug was administered to the subjects via the oral route. After three and six months of drug administration, blood samples were collected from each animal in the test groups and the haematological parameters were analysed and compared to the control group. Mean PCV was raised in 3months (35.09%) and 6months (39.27%) in subjects as compared with control (33.0%). Similarly, mean Hb increased significantly from 10.15g/dl in control to 11.28g/dl in subjects at 6 months ($p<0.05$). Mean RBC increased from $4.28x10^6$µl in control to $5.39x10^6$ µl in 3 months and $11.28x10^6$µl in 6 months ($p<0.05$). A similar trend was also observed in WBC and platelets. On the other hand, the mean MCV decreased significantly from 77.18fl in control to 65.18fl in subjects at 3 months and 66.55fl at 6 months ($p<0.05$). Also, the mean MCH and MCHC decreased significantly at 3 months and 6 months in subjects. The deviation from the control of most haematological parameters of albino rabbits after exposure to silver solution for six months found in this study is evidence that long-time exposure to silver solution significantly alters the haematological parameters of the exposed animals

Keywords: Heamtological parameters, Silver solution, Laboratory animals

INTRODUCTION

The antibacterial, antiseptic, and anti-tumour effects of silver have been established in many previous works (Elalfy *et al.* 2019) and have been widely used in diverse fields due to their superior properties (Nasir *et al.* 2015). In fact, Silver has been widely used as a powerful compound for disinfecting equipment, places and drinking water in the poultry industry (Rezaei *et al.* 2018). However, silver is a potentially toxic material that is used today in numerous consumer products (Imani *et al.* 2015) and it is believed to cause inflammation and toxicity (Gaiser *et al*. 2013). Silver nanoparticles, in particular, are used in a range of medical and consumer products because of its antibacterial activity (Raheem, 2018).

Raheem (2018) defined silver as a white shiny transitional metallic element found broadly in the human environment. He further explains that silver exists in little concentrations in the human body after a period of inhalation of the particles in drinking water and contaminated diet and air.

Due to the recent growing demand for silver because of its usage in medical, textile, cosmetics industries as well as its domestic use, there has been an increased fear as regards its safety and side effects in connection with its active silver iron Ag^+ in humans (Raheem *et al.* 2016). The cell level toxicity of silver colloids still remains unclear despite the previous reports of its harmful activities in vitro and in vivo (Mao *et al.* 2016; Elalfy et al. 2019). The maternal toxicity of silver nanoparticles has

also been reported (Adeyemiand Adewumi, 2014), its presence in the milk of female mice (Morishita et al. 2016) as well as its teratogenic effect on the skeletal malformation (Pani *et al.* 2015; Elalfy et al. 2019).

In a recent study on effects of silver nanoparticles on haematological parameters and hepatorenal functions in laying Japanese quails, Rezaei *et al.*(2018) found no effects of silver nanoparticles on their haematological parameters. In another study conducted on the effects of silver nanoparticles on haematological parameters of rainbow trout, *Oncorhynchus mykiss*, there were significant differences in the values of WBCs, HCT, Hb, RBCs, MCV, MCH, and MCHC between the treatments as compared to control (Imani et al. 2015). Similar findings were reported by Raheem (2018) in their study of the effects of silver nanoparticles on some blood parameters in rabbits and in another study on effects of intraperitoneally injected silver nanoparticles on histological structures and blood parameters in albino rat (Sarhan and Hussein, 2014).

The majority of the previous works focused on the toxicity effects of silver nanoparticles while the information onthe effects of colloidal silver solution on haematological parameters is rather scanty. The objective of this study is, therefore, to determine the effects of colloidal silver solution on haematological parameters,using laboratory animals as models.

MATERIALS AND METHOD

Study design

This experimental study involves 22 Albino rabbits that were obtained from the small animal unit of the National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria. The animals were grouped into 2 of 11 rabbitseach. Group one (subjects) was treated with 2.5ml Silver solution per day for six months and fed with commercially prepared rabbit pellets and clean water, while the group two (control) were fed with only commercially prepared rabbit pellets and clean water for six months. The drug was orally administered to the animals. The concentration of the colloidal silver solution was an engineered silver nano-particle mineral supplement 10 parts per million.

Sample collection

Blood samples (5mls) were collected from each rabbit through the marginal ear vein using the vacutainer set and placed in EDTA vacutainer tubes and analysed as the baseline data, blood samples were also collected from the test group three months and six months of treatment and analysed for some haematological parameters. The haematological parameters analysed include Packed cell volume (PCV), White cell count (WBC), Red blood cell count (RBC), Haemoglobin estimation (HB), Platelet count (Pt), Mean cell Haemoglobin concentration (MCHC), Mean cell volume (MCV), and Mean cell Haemoglobin (MCH).

Experimental protocol

A thin film of EDTA anticoagulated blood sample was made from the rabbit and was allowed to air dry and protected from dust and flies.The film was taken to the staining rack and was covered with Leishman stain, avoiding over floating and air bubbles and allowed to fix for 2 minutes. A volume of buffered distilled water, pH 6.8 twice the volume of the Leishman stain was used to dilute the stain and the slide allowed to stain for 8 minutes. The slide was washed with buffered distilled water, pH 6.8 and was air dried.The film was examined under the microscope using the X100 objective. The cells observed include; the Neutrophils, Lymphocytes, Eosinophils, Monocytes, Basophils, Platelets, and the general Red cell morphology, (Cheesbrough, 1998). An automated haematological analyser (Sysmex, Japan) was used to analyse the haematological indices of the EDTA blood samples from the rabbits before (baseline), between treatments (short term/3months) and after the treatment (long-term/6 months).

Statistical analysis

Data analysis was done using IBM SPSS for windows version 25.0. Analysis of Variance (ANOVA) was used to compare the measurements obtained between the subjects and control. Significance differences were judged for all analysis at $P<0.05$.

RESULTS

Table 1 shows variation of haematological parameters with the length of treatment with silver solution. The values of RBC, PCV, Platelets, WBC and heamoglobin were raised significantly with increase in the lenght of treatment with silver solution ($p<0.001$). Conversely, there were significant descrese in the values of MVC, MCH, and MCHC with the length of treatment with silver solution.

Table 2 shows the various haematological parameters of control, 3 months treatments and 6 months treatments with silver solution. The mean value of Parked cell volume (PCV) was higher after 3 months (35.09%) and 6 months (39.27%) treatment with colloidal silver solution as compared with control (33.0%). Similarly, the mean value of haemoglobin (Hb) significantly increased from 10.15 g/dl in control to 11.28 g/dl after 6 months of treatment ($p<0.05$). There wasa significant increase in the values of red blood cell (RBC) from $4.28x\ 10^6$ µl in the control group to $5.39\ x\ 10^6$ µl after 3 months and $11.28x\ 10^6$ µl after 6 months of treatment with the silver solution ($p<0.05$). A similar trend was also observed for white blood cell (WBC) and platelets.

Table 1: Correlation of heamtological parameters with the length of exposure to silver solution

Treated group	Correlation Coefficient (r)	P-Value
RBC ($x10^{12}$/l)	0.814^{**}	<0.001
PCV (%)	0.725^{**}	<0.001
Platlets($x10^9$/l)	0.714^{**}	<0.001
WBC($x10^9$/l)	0.665^{**}	<0.001
Haemoglobin (g/dl)	0.546^{**}	<0.001
MCV(fl)	-0.560^{**}	<0.001
MCH(pg)	-0.703^{**}	<0.001
MCHC(g/dl)	-0.712^{**}	<0.001

Table 2: Comparison of haematological parameters of control with treated group

Parameter	Control	3 months of treatment	6 months of treatment	P-value	limits
PCV (%)	33.00 ± 1.79	35.09 ± 2.34	39.27*[a] ± 3.17	<0.001	**33 – 50**
Hb (g/dl)	10.15 ± 0.46	10.68 ± 0.80	11.28* ± 0.90	0.005	**11 – 17**
RBC (10^6 µl)	4.28 ± 0.34	5.39* ± 0.37	5.86*[a] ± 0.61	<0.001	**4 – 6.2**
WBC (10^3µl)	4.72 ± 1.17	6.36* ± 1.78	7.69*[a] ± 1.26	<0.001	**4 – 12**
MCV (fl)	77.18 ±9.00	65.18* ± 3.12	66.55* ± 3.62	<0.001	**76.4 – 90.1**
MCH (pg)	23.91 ± 2.70	19.91* ± 0.70	19.27* ± 1.49	<0.001	**25.3-30.3**
MCHC (g/dl)	30.82 ± 0.60	30.36 ± 0.92	29.00*[a] ± 0.63	<0.001	**31 – 35.5**
Platelets (10^3 µl)	262.64 ±105.36	371.82* ± 44.77	498.55*[a] ± 127.69	<0.001	**150-400**

PCV = Packed cell volume, WBC =White cell count, RBC =Red blood cell count, HB = Haemoglobin estimation, Platelets = Platelet count, MCHC =Mean cell Haemoglobin concentration, MCV =Mean cell volume, MCH =Mean cell Haemoglobin. * Significantly different from control; a-significantly different from 3 months treatment.

On the other hand, there was a significant decrease in the Mean cell Haemoglobin concentration (MCV) from 77.18fl in control to 65.18 fl after 3 months and 66.55 fl after 6 months treatments ($p<0.05$).There was a steady decrease in the mean values of MCH and MCHC at 3 months and 6 months of administration of colloidal silver solution to rabbits in this study.

DISCUSSION

Exposure to a low dose of silver has been considered safe but recent studies have shown that long-time exposure or exposure to high doses of silver in a short time is harmful (Sarhan and Hussein, 2014). The objective of this study is to evaluate the effects of colloidal silver solution on the haematological parameters of albino rats orally exposed to 2.5 ml per day of the solution for3 months(short time) and 6 months (long-time exposure) respectively.

The study found that the mean PCV increased significantly after 6 months treatment as compared to 3 months and control (untreated group). The insignificant increase seen in the mean PCV after 3 months treatment might be due to short time treatment which was later raised due to high concentration of the silver solution in the bloodstream of the animals after 6 months exposure.Also, no statistically significant difference was observed in the mean values of Hb between 3 months treatments group and the control group. However, the mean value of Hb increased significantly after 6 months as compared to control. This finding contrasts the finding of Raheem (2018) who found that the mean haemoglobin decreased in rabbits immunized with 50µg/kg silver nanoparticles after thirteen days.

There were significant increase in the values of RBCs, WBCs, and platelets in both 3 months treatment and 6 months treatment groups as compared with the control group. Also, there were significantly higher values of these parameters in 6 months treatment group as compared to 3 months group. On the other hand, there was a significant decrease in the mean value of MCV after 3 months of treatment and a further decrease after 6 months treatment as compared to control group. Studies have also reported similar increase in the values of RBC and WBCs in groups treated with silver solutions (Raheem, 2018; Vandebriel et al. 2014) but our findings contrast the finding of Razavian and Masaimanesh (2015) who reported a decrease in WBC values in treated group and Rezaei et al. (2018) who did not find any changes in animals treated with silver nanoparticles.

Similarly, no statistically significant difference was observed in the mean values of MCHC between 3 months treatments group and the control group. At 6 months of exposure, the mean MCHC was decreased significantly as compared to both control and 3 months exposure. This is in agreement with findings of Raheem (2018) but contrast the (significant or non-significant) increased value reported by Imani *et al.* (2015) and no significant changes observed by Maneewattanapinyo et al. (2011).The variations found in various studies may be due to the difference in doses of silver solution administered and the duration of the study.

The mean values of PCV, RBC, and WBC were within the reference range after 3- and 6-months treatment whereas the mean haemoglobin after 3 months was lower than the reference limit. The mean values of MCV, MCH, and MCHC were lower than normal after 3 months and 6 months treatments but significant increase in the mean values of platelets were recorded in both 3 and 6 months of treatment with a colloidal silver solution. The values of PCV, RBC, WBC, MCHC, and platelets tend to change with longer treatment period as these parameters showed significant differences in 6 months as compared to 3

months treatment. The findings of this study are in agreement with a previous study which found significant differences in the values of WBCs, HCT, Hb, RBCs, MCV, MCH, and MCHC between the treatments as compared to control (Imani et al. 2015; Sarhan and Hussein, 2014) but contrast the finding of Rezaei et al. (2018) who found no effects of silver nanoparticles on the haematological parameters of Japanese quails administration in drinking water.

CONCLUSION

The deviation from the control of most haematological parameters of albino rabbits after exposure to silver solution for six months found in this study is evidence that long-time exposure to silver solution significantly alters the haematological parameters of the exposed animals. Therefore, we advocate caution against long time or unnecessary exposure to silver particles.

Ethical clearance

Ethical clearance was provided by the National Veterinary Research Institute, Federal Ministry of Agriculture and Rural Development, Plateau State with approval number AEC/03/85/20.

Conflict of interest

No conflict of interest declared.

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Nil

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