**Original article on**

**The Effects of ASAP (Colloidal Silver Solution) on Some Haematological Parameters Using Laboratory Animals as Models**

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Running Title: Effects of silver on heamatological parameters

Abstract

The objective of this study is to evaluate the effects of ASAP (colloidal silver solution) on the haematological parameters of albino rats 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 ASAP 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 administered to the subjects via the oral route. Blood samples were collected from each rabbit and haematological parameters were analysed to establish the baseline data. After three and six months of drug administration, 5ml of blood samples were collected from the test group and analysed for all the haematological parameters. Mean PCV was raised in 3-months (35.09%) and 6-months (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 4.28x106µl in control to 5.39x106 µl in 3 months and 6 months (11.28x106µl) (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 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 ASAP solution for six months found in this study is evidence that long-time exposure to ASAP significantly alters the haematological parameters of the exposed animals.

**Keywords:** Normocytic Normochromic, Haemoglobin, Macrocytic Anaemia, Hyperchromasia, Haemopoeitic Organs, Argyria

**Introduction**

The antibacterial, antiseptic, and anti-tumour effects of silver have a been established in many previous works (Elalfy, Abdraheem, & Abouelmagd, 2019) and have been widely used in diverse fields due to their superior properties (Nasir, Alaa, & Mohammed, 2015). In fact, Silver has been widely used as a powerful compound for disinfecting equipment, places and drinking water in the poultry industry (Rezaei, Farzinpour, Vaziry, & Jalili, 2018). However, silver is a potentially toxic material that is used today in numerous consumer products (Imani, Halimi, & Khara, 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 over a period of inhalation of particles in the drinking water and contamination of the diet and air (Raheem, 2018).

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, Al-Thahab, & Abd, 2016). The cell level toxicity of silver colloids still remains unclear despite the previous reports of its harmful activities in vitro and in vivo (Elalfy et al., 2019; Mao, Tsai, Chen, Yan, & Wang, 2016). The maternal toxicity of silver nanoparticles has also been reported (Adeyemi & Adewumi, 2014), its presence in the milk of female mice (Morishita et al., 2016) as well as its teratogenic effect on the skeletal malformation (Elalfy et al., 2019; Pani *et al.,* 2015).

In a recent study on effects of silver nanoparticles on haematological parameters and hepatorenal functions in laying Japanese quails, Rezaei *et al.* found no effects of silver nanoparticles on the haematological parameters of Japanese quails administration in drinking water (Rezaei et al., 2018). 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 the albino rat by Sarhan & Hussein (2014).

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

**Materials and method**

An automated haematological analyser (Sysmex) 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). Film were made and stained with Leishman

**Thin-film preparation**

A drop of the EDTA anticoagulated blood sample from the rabbit was placed towards the end of a clean grease-free slide, about 1cm from the end, without delay a spreader was placed in front of the drop of the blood at an angle of 300 and moved backwards until it made a contact with the drop of blood. A quick spread was made with a steady movement to spread the blood along the slide to produce a thin film. The thin film was allowed to air dry and protected from dust and flies (Monica Cheesbrough, 1998).

**Staining of thin blood film**

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, (Monica Cheesbrough, 1998).

**Experimental design**

ASAP (colloidal silver solution) was consistently administered to the test group consisting of eleven rabbits at 2.5ml/day for six (6) months through the oral route. The concentration of the colloidal silver solution, ASAP was an engineered silver nano-particle mineral supplement 10 parts per million.

**The subjects**

Twenty-two (22) Albino rabbits 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 rabbits. Group 1 consisted of11 rabbits treated with ASAP Colloidal Silver Solution 3 times daily for 6 months while Group 2 (the control group) consisted of 11 rabbits fed only with commercially prepared rabbit’s pellets and water for 6 months. The drug was orally

**Sample collection**

Blood samples were collected from each rabbit through the marginal ear vein using the vacutainer set. Blood samples (5mls) were collected in EDTA vacutainer tubes from all the rabbits before treatment and analysed as the baseline data, blood samples were also collected from the test group three months after and then six months after treatment for some haematological analysis. 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).

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 trimesters. Significance differences were judged for all analysis at P<0.05.

**Results**

Table 2 shows the various haematological parameters of control, 3 months treatments and 6 months treatments. The mean value of Parked cell volume (PCV) was higher after 3 months (35.09%) and 6 months (39.27%) treatment with ASAP (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 was a significant increase in the values of red blood cell (RBC) from 4.28x 106 µl in the control group to 5.39 x 106 µl after 3 months and 11.28x 106 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.

On the other hand, there was a significant decrease in the Mean cell Haemoglobin concentration (MCV) from 77.18 fl 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 ASAP (colloidal silver solution) to rabbits in this study.

Table 3 shows the comparison of mean haematological parameters of laboratory rabbits treated with a colloidal silver solution for 3 months and 6 months with normal limits. The mean value of PCV increased from 33.0% for control to 35.09% after 3 months of treatment and later to 39.27% after 6 months of treatment. A similar trend was observed for RBC and WBC but these values were within the normal limits. The mean values of MCV, MCH, and MCHC were lower than the normal limits after 3 months and 6 months treatments as compared to the control group. On the other hand, the mean platelet was within normal limits in control (262.62 x 109/l) and the 3 months treatment group (371.82 109/l) but higher than normal limit among the 6 months treatment group (498.55 109/l).

**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 & Hussein, 2014). The objective of this study is to evaluate the effects of ASAP (colloidal silver solution) on the haematological parameters of albino rats orally exposed to 2.5 ml per day of the solution for 3 months (short time) and 6 months (long-time exposure).

The study found that the mean PCV increased significantly after 6 months treatment as compared to 3 months and control (untreated group); p<0.05. 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 ASAP 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 though there was a slight increase of Hb in after 3 months exposure than in control (p>0.05). However, the mean value of Hb increased significantly after 6 months as compared to control (p<0.05). 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 was a significant increase in the value of RBCs, WBCs, and platelets in both 3 months treatment and 6 months treatment groups as compared with the control group (p<0.05). Also, there were significantly higher values of these parameters in 6 months treatment group as compared to 3 months group (p<0.05). 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 (p<0.05). This finding is similar to the finding of previous studies who found that RBC increased in groups treated with silver solutions while WBC remained within the normal limit (Raheem, 2018; Razavian & Masaimanesh, 2015; Vandebriel et al., 2014) but contrast the report of Imani *et al.* (2015) that WBCs was higher in all treatments than the control group and the report of Rezaei et al., (2018) who did not find any changes in haematological parameters of in laying Japanese Quails 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 though there was a slight decrease in MCHC in 3 months treatment group as compared to the control group (p>0.05). At 6 months of exposure, the mean MCHC was decreased significantly as compared to both control and 3 months exposure (p<0.05). This is in agreement with findings of Raheem (2018) but contrast the 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 administered and the duration of the study.

The mean values of PCV, RBC, and WBC were within the normal range after 3- and 6-months treatment whereas the mean haemoglobin after 3 months was lower than the normal 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 (p<0.05). 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 & 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 ASAP solution for six months found in this study is evidence that long-time exposure to ASAP (colloidal silver solution) significantly alters the haematological parameters of the exposed animals. Therefore, we advocate caution against long time or unnecessary exposure to silver particles. Also, further studies should consider the effect of colloidal silver on the Novel Corona Virus (Covid-19).

**Ethical clearance**

Ethical clearance was provided by the National Research Institute, Vom, Plateau State.

**Conflict of interest**

No conflict of interest declared.

**Acknowledgements**

Nil

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administered to the rabbits 3 times daily according to their body weight for 6 months.

**Table 1: Experiment groups:**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| GROUP 1: TEST (T) | | | | | | | | | | | | |
| Rabbits I. D | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | |
| Body Weight (kg) | 1.4 | 1.4 | 1.6 | 1.5 | 1.4 | 1.6 | 1.5 | 1.9 | 1.4 | 1.4 | 1.5 | |
| Temp. (0C) | 39.5 | 40.2 | 39.0 | 39.6 | 39.3 | 37.8 | 38.7 | 39.8 | 39.2 | 40.6 | 37.1 | |
| Route | Oral | Oral | Oral | Oral | Oral | Oral | Oral | Oral | Oral | Oral | Oral | |
| Treatment | ASAP | ASAP | ASAP | ASAP | ASAP | ASAP | ASAP | ASAP | ASAP | ASAP | ASAP | |
| Dosage (ml/kg/day) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | |
| GROUP 2: CONTROL (C) | | | | | | | | | | | | |
| Rabbits I. D | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | | C11 |
| Body weight (kg) | 1.2 | 1.5 | 0.9 | 1.4 | 1.0 | 1.4 | 1.3 | 1.4 | 0.9 | 1.2 | | 1.4 |
| Temp. (0C) | 39.5 | 38.4 | 37.2 | 36.9 | 39.1 | 39.0 | 35.9 | 37.6 | 39.7 | 36.4 | | 38.0 |
| Treatment | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | | Nil |

**Table 2: Comparison of haematological parameters of control with treatment groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Control | 3 months of treatment | 6 months of treatment | P-value |
| PCV (%) | **33.00 ± 1.79** | **35.09 ± 2.34** | **39.27\*a ± 3.17** | **0.000** |
| Hb (g/dl) | **10.15 ± 0.46** | **10.68 ± 0.80** | **11.28\* ± 0.90** | **0.005** |
| RBC (106 µl) | **4.28 ± 0.34** | **5.39\* ± 0.37** | **5.86\*a ± 0.61** | **0.000** |
| WBC (103µl) | **4.72 ± 1.17** | **6.36\* ± 1.78** | **7.69\*a ± 1.26** | **0.000** |
| MCV (fl) | **77.18 ±9.00** | **65.18\* ± 3.12** | **66.55\* ± 3.62** | **0.000** |
| MCH (pg) | **23.91 ± 2.70** | **19.91\* ± 0.70** | **19.27\* ± 1.49** | **0.000** |
| MCHC (g/dl) | **30.82 ± 0.60** | **30.36 ± 0.92** | **29.00\*a ± 0.63** | **0.000** |
| Platelets (103µl) | **262.64 ±105.36** | **371.82\* ± 44.77** | **498.55\*a ± 127.69** | **0.000** |

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.

**Table 3: Comparison of haematological parameters of treatment groups with normal limits**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Control | 3 months of treatment | 6 months of treatment | Normal limits |
| PCV (%) | **33.0** | **35.09 normal** | **39.27 normal** | **33 – 50** |
| Hb (g/dl) | **10.15** | **10.68 low** | **11.28 normal** | **11 – 17** |
| RBC (x1012l) | **4.28** | **5.39 normal** | **5.86 normal** | **4 – 6.2** |
| WBC (x 109/l) | **4.72** | **6.36 normal** | **7.69 normal** | **4 – 12** |
| MCV (fl) | **77.18** | **65.18 low** | **66.55 low** | **76.4 – 90.1** |
| MCH (pg) | **23.91** | **19.91 low** | **19.27 low** | **25.3-30.3** |
| MCHC (g/dl) | **30.82** | **30.36 low** | **29.00 low** | **31 – 35.5** |
| Platelets (x109/l) | **262.64** | **371.82 normal** | **498.55 high** | **150-400** |