

## RESEARCH ARTICLE

# Effect of argentum nitricum ( $\text{AgNO}_3$ ) in both *in vivo* and *in vitro* systems

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### ABSTRACT

Homeopathy is one of the branches of medicinal biology, serving the mankind over more than two centuries, and is still rare to implicate in research field. The present approach is to use this cosmos to check its effects and side effects (if any) which may or may not cause any change in prokaryotic and eukaryotic system. For this it can be considered one well known homeopathic medicine for study viz. Argentum nitricum or silver nitrate ( $\text{AgNO}_3$ ) which is an analogue of allopathic medicine 'Cetrizine'. Homeopathic medicines are available in different homeopathic potency (dilutions), but for the experimental purpose, it has took into consideration crude extract (mother tincture), a safe dose (potency 30) and a higher dilution 200 of the above. Assessment of the effects of medicine was performed on two systems namely, *in-vivo* (in body) and *in-vitro* (in laboratory). In *in vitro* system, the study was done by considering bacteria (gram positive, gram negative) and fungi, their growth under normal condition and growth after homeopathic medicine treatment. Also molecular analysis was done by checking out the central dogma pathway. Whereas in the *in vivo* system, dose effectiveness was checked in eukaryotic body (genetically pure line albino rats) and the various physiological parameters were studied in relation to the interrelated organs.

**Key Words:** Bacterial and fungal growth, central dogma, eukaryotic body, homeopathic potency.

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### INTRODUCTION

The main motive of the research work was to study the effectiveness and standardize efficacious mode of Homeopathic medicine in detoxification mechanism on Eukaryotic system by *in-vitro*, *in-vivo* and molecular analysis. It was also wanted to reveal the different changes in phenotypic characters and physiological parameters. Homeopathy is a system of alternative medicine developed by Samuel Hahnemann in 1796, based on his doctrine of "like cures like", according to which a substance that causes the symptoms of a disease in healthy people will cure similar symptoms in sick people. Homeopathy is often criticized for having little research evidence available. It uses many animal, plant, mineral, and synthetic substances in its remedies. One of the major reasons that the results of most mainstream research on homeopathy are often inconclusive because the methods used usually do not honor the principles of homeopathy. Unfortunately, remedies are often not prescribed individually and are instead selected based on typical clinical presentation of pathology. "Classical homeopathy was defined as comprehensive homeopathic history-taking, followed by the prescription of a single individualized remedy, possibly with subsequent change of remedy in response to changing symptoms. If no comprehensive homeopathic history was taken and all patients received a single, identical remedy, interventions were classified as clinical homeopathy". In producing remedies for diseases, the process used is called "potentization", whereby a substance is diluted with alcohol or distilled water and then vigorously shaken by 10 hard strikes against an elastic body in a process called "succussion". Accepting that homeopathic medicines have a biological effect, even in potencies where dilution goes beyond Avogadro's number, the point at which not a single molecule of the original substance is likely to remain in the solution is not easy.

Argentum nitricum, also known as silver nitrate ( $\text{AgNO}_3$ ), is an inorganic compound that is used as an antiseptic medicine because of its antiseptic properties. The common name of Argentum nitricum is Hell Stone or Devil's Stone. It is prepared by dissolving Silver Nitrate in alcohol to form the mother tincture. The medicinal use of Silver Nitrate dates back to the middle Ages when it was used as an antiseptic and caustic to treat wounds. Today silver nitrate is still used in wart treatments and although not common. Mild poisoning still occurs from treating warts, giving some idea of the toxicity of this compound. Symptoms of mild poisoning are anxiety and agitation, originally proved by Hahnemann and again by Muller in 1845.

Most important action is on the blood, producing an antiplastic effect, rendering it more fluid and darker; the red corpuscles lose their coloring matter; the blood stagnates in the vessels and nutrition is interfered with. The nerve centers are also involved, giving rise to various symptoms of cerebral and spinal derangement. Silver nitrate is applied for removing various types of disorders like nervousness, apprehensions, digestive disorders, asthma and anxiety. Its potency lies in the range 6c to 200c. The particular therapeutic usage of this homeopathic remedy perfectly fit for the middle ages whenever it is used as an antiseptic along with corrosive to deal with injuries.

### MATERIALS AND METHODS

#### Isolation of genomic DNA from bacterial and fungal sources

Gram positive-*Bacillus sp.* (MTCC-2497) and Gram negative - *E. coli* (BL-12) were used as bacterial strains and were grown overnight in LB broth were transferred in a micro centrifuge tube and centrifuged. The pellet was resuspended in TE buffer (Tris-EDTA, pH 8.0). 10% SDS was added and incubated. Phenol-chloroform mixture (1:1) was added and mixed. The mixture was centrifuged at 10,000 rpm. The upper aqueous phase was transferred into a new tube and re-extracted by equal volume of phenol-chloroform. The mixture was centrifuged again at 10,000 rpm. The upper aqueous was taken and mixed with 3M sodium acetate (pH 5.2) and isopropanol was added and mixed gently to precipitate the DNA. Again centrifugation was performed at 10,000 rpm to precipitate the DNA. The DNA was washed by 70% ethanol for 30 seconds and centrifuged briefly. The DNA was re-suspended in TE buffer.

The fungal broth (YEPD) was taken and 0.5M suspension buffer (Tris Chloride, pH-7.5) was mixed along with sand particles and kept in ice for 15 seconds. 1% SDS was added and incubated. The tubes were centrifuged and phenol-chloroform mixture (1:1) was added followed by centrifugation. 3M sodium acetate (pH 5.5) was added to the supernatant followed by immediate addition of absolute alcohol. Then it was placed in the freezer at -20°C for 30 mins. After cooling, centrifugation was done and the pellet was washed with 70% ethanol. Finally it was suspended in TE buffer.

**Agarose gel electrophoresis of DNA:**

Agarose gel (0.8%) was prepared by dissolving in 1X TAE buffer. The comb was inserted before pouring the gel. The gel was left for 30-40 minutes for solidification and comb was removed from the gel. Electrophoresis tank containing the gel was filled with 1X TAE electrophoresis buffer. 1 volume of sample buffer was mixed with the 5 volume of DNA sample. The samples were applied to the wells formed in the gel. Ethidium bromide at a concentration of 0.5µg/ml was incorporated into the gel. The electrodes were connected and 70 volt current was applied for 1 hour. After electrophoresis, the gel was visualized under UV light.

**Sodium dodecyl sulfate (SDS)-gel electrophoresis:**

SDS-gel electrophoresis was done according to the methodology followed by Konar *et al.* (2013). For this, the SDS plates were assembled. Acrylamide separating gel was prepared in a conical flask and poured between the plates. Acrylamide stacking gel was prepared. The components were mixed and poured over the separating gel. Wells were prepared using combs. The samples were prepared with equal volume of sample buffer. The samples were then heated in boiling water bath and loaded. Voltage was applied depending upon the dimension of gel. The gel was allowed to run until the sample reaches the bottom of the resolving gel. The power supply was then turned off; the gel was removed from the apparatus. The gel was allowed to stain overnight. Next day, the gel was dipped in destaining solution and observed.

**In vivo work:**

Four albino rats were taken and were treated with alcohol for the control set, argentum nitricum Mother, argentum nitricum potency-30, argentum nitricum potency-200. After 60 days, they were sacrificed and different physiological parameters such as presence or absence of cyst, change in size of the organs and presence of any other abnormality in liver, heart, spleen and kidney were studied (table 1). Their phenotypic and hematological parameters were studied. The blood was taken from the heart on a slide and was stained with Leishmann stain, washed with water and observed under the microscope.

Table 1. In *in vivo* condition, four albino rats were taken; different physiological parameters and effects in different Homeopathic potency were studied

Sl. no.	Treatment time (days)	Types of treatment	Dose	Initial weight (g)	Weight after 30 days (g)	Weight after 60 days (g)	Physiological parameter studied and changes observed after treatment.			
							Liver	Kidney	Spleen	Heart
1.	60	Water	Water 50ml/day	131.8	144.32	137.6	Normal	Normal	Normal	Normal
2.	60	AgNO <sub>3</sub> - 200	10 drops in 50ml water	190.3	189.75	193.3	Big cyst	Normal	Normal	Normal
3.	60	AgNO <sub>3</sub> - 30	10 drops in 50ml water	178.3	162.2	186.25	Small cyst	Normal	Small size	Normal
4.	60	AgNO <sub>3</sub> Crude	10 drops in 50 ml water	98.20	98.72	110.24	Normal	Normal	Small size	Normal

**RESULTS AND DISCUSSION**

Three results were observed in *in vitro* condition, i.e., bacterial growth, bacteria and homeopathy medicine, fungi and homeopathy medicine. When bacteria grow normally, there was an increase in optical density value with respect to time. When bacteria grow with homeopathy medicine, optical density mentioned in table 2 gets decreased as homeopathic potencies inhibit the growth of bacteria so decline in the optical density value was observed in gram negative bacteria whereas gram positive bacteria showed gradual increase. Central dogma was studied and presence (+) or absence (-) of band of DNA (agarose gel electrophoresis) and protein (SDS-PAGE) is mentioned in the table 3.

Table 2. Optical density at 400 nm of cultures of bacteria and fungi

Duration of treatment	Fungi			Gram positive bacteria ( <i>Bacillus 2479</i> )			Gram negative bacteria ( <i>E. coli. BL-121</i> )		
	200	30	Crude	200	30	Crude	200	30	Crude
1 <sup>st</sup> hour	0.02	0.00	0.04	0.00	0.01	0.03	0.10	0.05	0.03
2 <sup>nd</sup> hour	0.03	0.03	0.01	0.19	0.20	0.20	0.07	0.08	0.06
3 <sup>rd</sup> hour	0.01	0.20	0.03	0.24	0.25	0.29	0.01	0.01	0.02
4 <sup>th</sup> hour	0.01	0.02	0.06	0.28	0.29	0.31	0.01	0.05	0.02
5 <sup>th</sup> hour	0.01	0.03	0.02	0.29	0.29	0.29	0.00	0.04	0.02

6 <sup>th</sup> hour	0.02	0.02	0.04	0.25	0.29	0.33	0.02	0.02	0.01
7 <sup>th</sup> hour	0.03	0.02	0.03	0.28	0.27	0.37	0.05	0.01	0.00

Table 3. Result of agarose gel electrophoresis and SDS-PAGE

Type	Gram Positive		Gram Negative		Fungi	
	DNA	Protein	DNA	Protein	DNA	Protein
Control	+	-	+	-	-	-
Argentum nitricum crude extract	+	-	+	-	+	+
Argentum nitricum Potency 30	-	-	-	-	+	-
Argentum nitricum Potency 200	+	-	-	-	+	-

From figure 1, it can be concluded that the homeopathic medicine did not have any significant effect on the growth of fungi, since their growth remained static. The graph initially moved upward because of normal growth of bacteria/fungi then it decline (bactericidal/fungicidal) or be in a stationary phase because of Homeopathic potency.

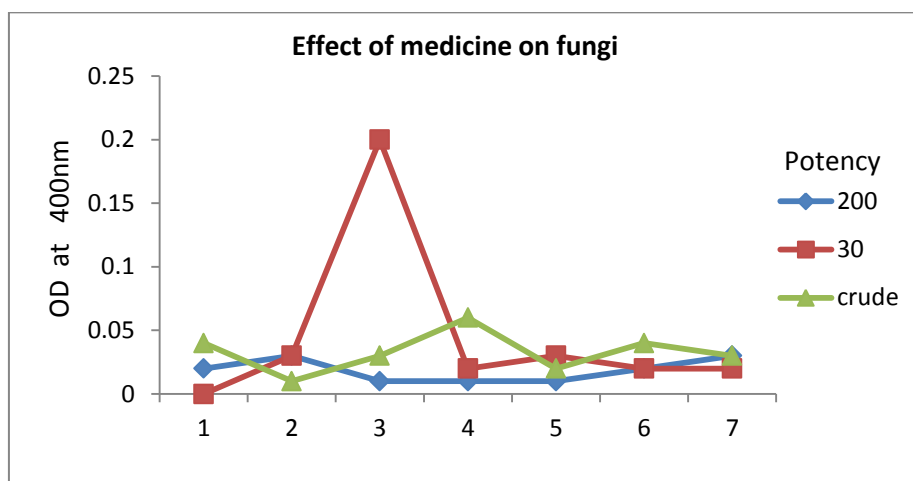


Figure 1. Effect of Argentum nitricum on fungi

Surprisingly the homeopathic medicine did not inhibit the growth of the gram positive bacteria as they show their normal growth activity with increase in optical density (figure 2). On the other hand, inhibition of growth of gram negative bacteria by the homeopathic medicine was observed indicated by decline in the optical density (figure 3).

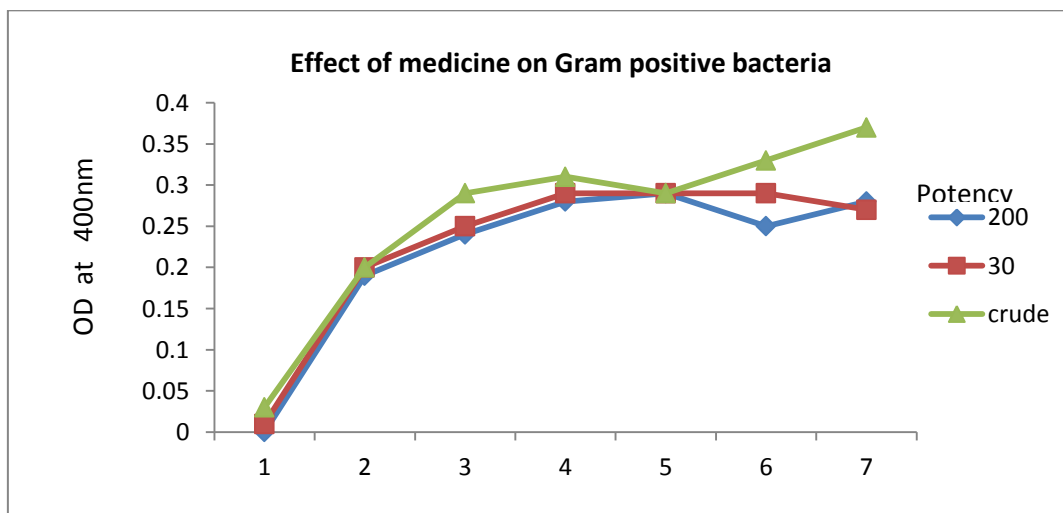


Figure 2. Effect of Argentum nitricum on gram positive bacteria

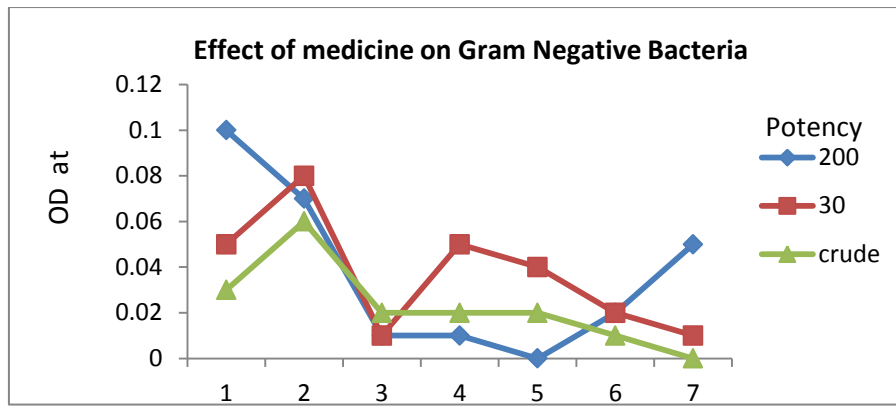


Figure 3. Effect of Argentum nitricum on gram negative bacteria

Microscopic view of blood film showed that the cells, those were treated with argentum nitricum with potency-200, have a shrinkage and the rest of them showed no significant change (figure 4).

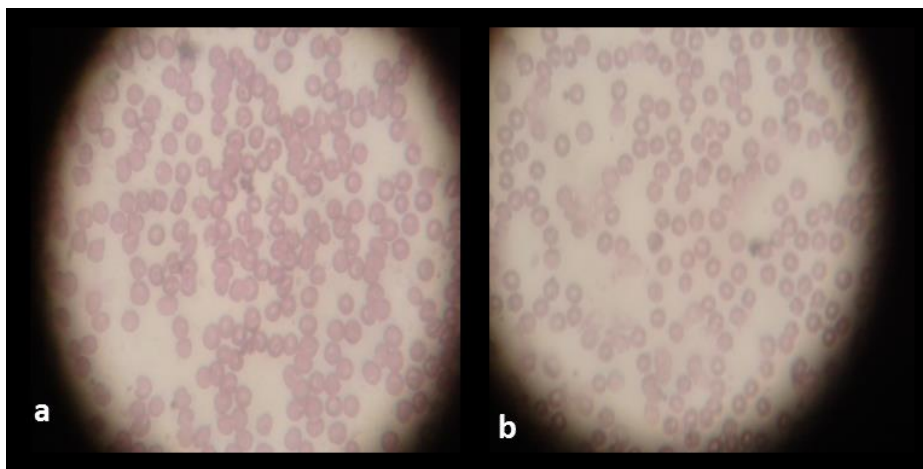


Figure 4. Microscopic slide view; (a) blood film slide for control, (b) blood film slide of AgNO<sub>3</sub>-200

Treatment of mice with argentum nitricum led to formation of cyst in the liver (figure 5). The mice treated with higher dilution of medicine (potency – 200) showed more number of cyst because it was more readily absorbed by the cells of its body as compared to the medicine with lower dilution (potency – 30).



Figure 5. Mice dissection; (a) single cyst in liver of mice treated with AgNO<sub>3</sub> of potency 30 (b) double cyst in mice liver treated with AgNO<sub>3</sub> of potency – 200.

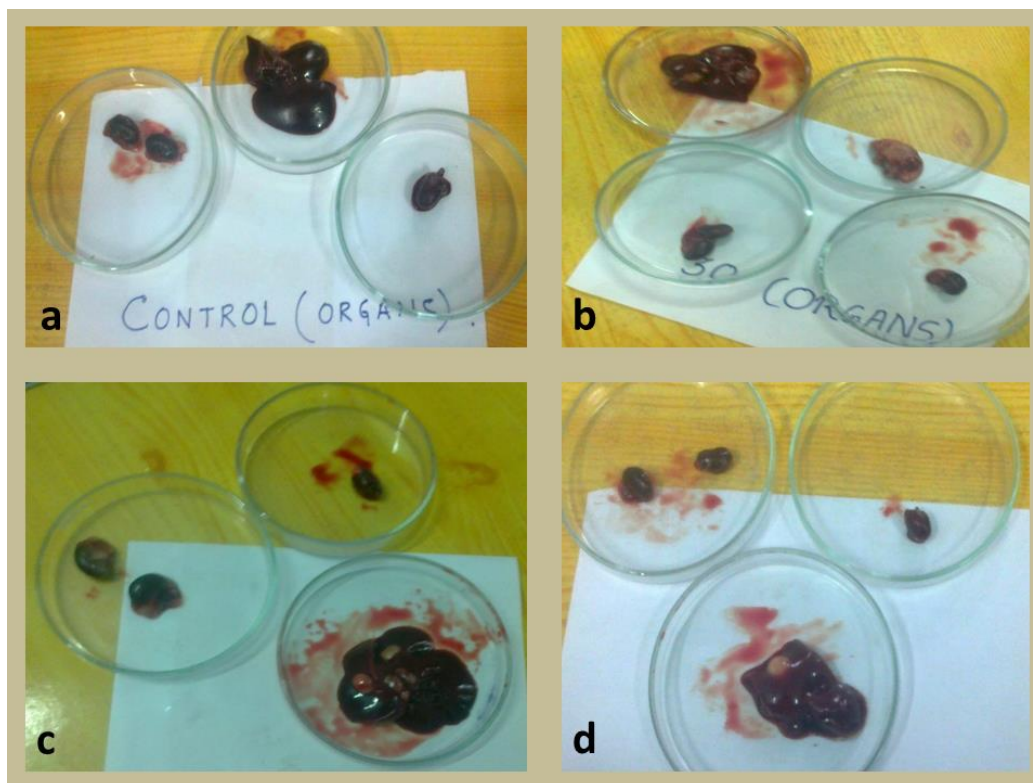


Figure 6. Histological view; (a) mice organs were used as control, (b) mice organs were treated with  $\text{AgNO}_3$ -30, (c) by  $\text{AgNO}_3$ -200 treatment, and (d) treated with crude  $\text{AgNO}_3$

#### CONCLUSION

Aim of the experiment was to study the effect of Homeopathic medicine argentum nitricum on eukaryotic and prokaryotic system. Both *in vitro* and *in vivo* showed detoxification of Homeopathic potency. But gram positive bacteria showed continuous increase in optical density with time due to certain unknown reasons. Also in *in vivo* condition, some physiological changes were observed such as decrease in size of spleen and formation of cyst was found in the treated albino against the untreated. Some phenotypic changes were also observed like hair fall and sudden change in weight. As we all know that Homeopathic medicine has no side effects but from this experiment we can conclude that Homeopathic medicine is not safe for long term application.

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