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# **Past mastering of metal transformation enabled physicians to increase their therapeutic potential**

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## **Abstract**

*Background:* metals are trace elements, vital in some instances or toxic in others. Due to this toxicity, they have been used since ancient time as antimicrobials, and prescribed when plant-only remedies were not efficient enough. These remedies could still contain secrets that may lead to the discovery of new therapeutically interesting combinations. The objective of this study was to give a proof of concept that such remedies combining metals and plants are worth studying again.

*Methods:* we exploited 4 medical formularies (*aqrābādhīn*), from three Arab authors from the 9-12<sup>th</sup> century. We reproduced a remedy, and analyzed the role of each ingredient. We further looked for the minimum inhibitory concentration against three pathogenic bacteria, and we analyzed toxic and inflammatory effects of this remedy on macrophages.

*Results:* even if plants were extensively used (almost 80% of all ingredients), more than 36 different minerals have been found in these 4 *aqrābādhīn*. When it came to remedies against infections that could be applied externally, the use of metals grew to 70%. We focused on a remedy, containing mainly metals. We have been able to attribute a role for each ingredient, to show that this skin remedy helped to combat the infection and to resorb the wound, and to highlight the mastering of metal transformation by these physicians.

*Conclusions:* with a very simple recipe, mainly composed of metals, these past physicians designed a complete and synergistic remedy to combat abscesses, while restricting the toxic effect of metals to the site of infection. It is a first example showing that different metal manufactures were evolved to improve their therapeutic potentials. The knowledge acquired by these physician should deserve more attention, and unexpected features, original organo-metallic compounds or therapeutic synergy could still be found from such research.

## 1. Introduction

Some metals are fundamental for many cellular functions in all living organisms. About half of the proteins are dependent on metals for structure, function, electron transfer, catalysis properties [1] [2]. These essential metals are lethal to any organism when present in excess, mostly due to the generation of reactive oxygen species (ROS), or their competition with other essential metals. Furthermore, some metals like gold (Au), silver (Ag), mercury (Hg), lead (Pb), arsenic (As) are extremely poisonous to most organisms, including bacteria even at low concentration. Because of their toxicity, these metals have been used since ancient time as antimicrobial agent, to preserve food or liquid. For example, vessels made of copper (Cu) and Ag have been used for centuries to disinfect water or preserve food. Metals have also been widely utilized since Antiquity in remedy, as anti-infective molecules. The Edwin Smith Papyrus, an ancient Egyptian medical text dating back to 1,600 B.C., is one of the first written works that documents the use of a Cu salt as an astringent [3], [4]. Many applications of Ag in medicine have been documented, to treat gonococcal ocular infection [5], repair vaginal tears after childbirth [6], prevent infection of surgical wounds etc. Physicians have also used tellium (Te), As oxides, Cu or Hg salts to treat diseases such as leprosy, tuberculosis, gonorrhoea and syphilis [7], [8], [9], [10], [11]. Pb has been used for centuries in remedies, food preservation, sweetenend, as well as in make-up [12], [13]. The use of metals has been abandoned after the discovery of penicillin by Alexander Fleming in 1920. Since then, antibiotics were preferred as they were powerful, their mode of action well described and their toxicity better handled. Certain unfortunate and inappropriate use of metals have further largely contributed to the current mistrust concerning their use in medicine [14], [15], [16], [17]. However, the spread of resistant strains and limitations in the development of new antibiotics has led to a renewed interest in the use of metals [18]. Due to their constant use over centuries, their anti-microbial properties are proven and admitted and they could become attractive again as long as their effect

on patients are scientifically validated. Studies on past use of metals could provide some valuable informations concerning their use, precautions to be taken or warning on contraindications. Indeed, even if metals were widely employed, they were neither used randomly, nor without transformation or mastering their sources, their purity and their formulation.

The Middle Age Arabic period corresponds to a golden scientific apex with crucial innovations in medicine. The knowledge of the Arab physicits was not based on a form of empiricism but anchored in a thousand-year-old theory, the theory of humor which was current until the advent of molecular medicine beginning of the 19th century. The theory of humor is a world of its own, well organized, in which everything has a place and an explanation. Numerous studies show the effectiveness of ancient drugs and this medicine was based on strong knowledge of the properties of natural elements, such as plants, minerals or animal materials made available to humans to treat their fellow human beings [19], [20], [21], [22]. The Arabs developed an advanced system of care characterized by particularly rich and abundant sources, which exist under different types of documents including medical formularies or *aqrābādhīn* listing hundreds of drug preparations (illustrated Figure 1).

The challenge of this multidisciplinary research based on the study of ancient knowledge is to find out whether these ancient pharmacopoeias may contain solutions that could be implemented for the design of tomorrow's anti-infectious drugs.

The objective of this study was to give a proof of concept that these past physicians did not choose metals randomly, took into account their toxicity in their practises, and mastered sufficiently the transformation of these metals to confer them specific properties, depending on the disease to treat. Through a historical survey, we listed the metals used in the past, and highlighted the ones used to cure different affections referring to symptoms related to infections. By exploring this historical literature, it appeared that metals were frequently used

in this pharmacopeia for cutaneous infections and rarely ingested. We then focused on one simple remedy, an ointment that was used to cure abscesses. This remedy contained litharge (PbO), and although the choice of this metal for the development of an application nowadays seems inappropriate due to its well-known toxicity, some scientific arguments still plaid for its study as a proof of concept. Due to its wide use in the past, it may represent a good illustration of the ability of past physicians to handle the sources, the transformation, the properties, the use and the toxicity of metals. We have been able to reproduce this remedy, identified the steps important to get the ointment, and studied its biological activity by looking for its anti-bacterial properties and its effect on the inflammation system. It appeared that this simple combination of metals might enable to combat the infection, and to resorb the wound. Further, our results showed that by mastering the transformation of litharge, physicians could confer specific and different properties to metals according to the recommended use, and were able to restrict their toxic effects.

## 2. Materials and Methods

### 2.1 Selection of sources

We exploited 4 *aqrābādhīn* i.e. medical formulary, from three authors, using double editions in translated versions or Arabic-written versions. The authors of the Arabic manuscripts lived from the 9th and the beginning of the 12th century. Their biographies are briefly described hereafter:

- Sābūr ibn Sahl *aqrābādhīn* exists in two versions, the small dispensary [23] which is a smaller version of an extended manuscript prepared for use by pharmacists at Adudi Hospital in Baghdad [24]. He was one of the first doctor to write a pharmacopoeia text to the intention of doctors exercising in an hospital, describing a wide variety of drugs and measures to be taken to cure ailments. He became a doctor at the court of Caliph al-Mutawakkil (847-869) and his successors until his death in 869 (date uncertain).

- Al-Kindī (800-870 A.D.) wrote an *aqrābādhīn* [25] He was born at the beginning of the 9th century in Kufa. In the pharmaceutical field, for the first time he established mathematical rules to determine the final effect of a compound remedy, starting from the quantity and the degrees of quality of each ingredient, thus becoming "the father of dosage".

- Al-Tilmīdh (Baghdad, 1073 – 1165 A.D.) is a doctor of Baghdad. He was notably at the service of the Abbasid caliphs al-Muqtafi and al-Mustanjid, chief of the doctors of Baghdad, head of the hospital 'Adudi. His experience in this establishment led him to write a twenty-chapters *aqrābādhīn* compiled according to the author from a number of other *aqrābādhīnāt* [26].

### 2.2 Chemicals, bacterial strains, eucaryotic cell lines, and growth conditions

For the reproduction of the remedy, litharge ( $\text{Pb}^{\text{II}}\text{O}$ ) has been purchased from Strem Chemicals, Celite 545 from VWR Chemicals, red vinegar, olive oil, sheep fat, quicklime

(CaO) from commercial market. Tobramycin was purchased from Sigma. The bacterial strains tested were *Pseudomonas aeruginosa* ATCC15692; *Escherichia coli* MG1655; *Staphylococcus aureus* HG001. These strains were routinely grown in LB (Difco) at 37°C. When Succinate Medium (SM) was used (per liter: K<sub>2</sub>HPO<sub>4</sub> 6 g, KH<sub>2</sub>PO<sub>4</sub> 3 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.2 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1 g, succinic acid 4 g, pH adjusted to 7.0), the bacteria were first grown overnight at 30°C in LB broth and were then washed, re-suspended and cultured overnight at 37°C in SM medium. The cells were then pelleted by centrifugation and re-suspended in fresh medium, and the resulting suspension was diluted to the OD<sub>600nm</sub> required. RAW 264.7 mouse macrophages (ATCC® TIB-71™) were cultured in Dulbecco's Modified Eagle Medium High glucose (DMEM High Glc) containing stabilized glutamine, supplemented with 5% (v/v) of fetal bovine serum (FBS), Penicillin (1000 U/mL) and Streptomycin (100 µg/mL) at 37°C in 5% CO<sub>2</sub>, 95% humidity. Media and culture supplements were from Sigma/Merck (Darmstadt, Germany). For ICP-AES, TraceMetal grade HNO<sub>3</sub> 67-69 % was purchased from Fischer Chemical, and element standards were purchased from CPI international.

### 2.3 Preparation of the remedy

10 g of celite 454 were placed in a syringe plugged with cotton. Then, 1 g of quicklime and 1 g of litharge were added. Vinegar was then poured onto the syringe until 50 mL of filtrate was obtained (as shown in Figure 2, top), the filtrate was recovered in a round-bottom flask. 50 ml of olive oil was added as well as 4 g of sheep fat and the preparation was heated at 150°C with a heating mantle for 1 h, then passed to the flame (Figure 2, bottom). Depending on the experiment, one or more of the ingredients/steps could be omitted. To test the antibacterial and inflammatory properties of the preparations, we did neither heat nor added sheep fat and olive oil, to avoid testing either poorly soluble materials or aggregates that could bias the results.



#### 2.4 Metal content analysis

Samples were mineralized in closed vessels with nitric acid for trace analysis at 100°C for 1h. A blank test was carried out in parallel under the same conditions. The detection of 30 elements in the clear obtained solution was realized at the proper dilution by ICP-AES (Varian 720ES). Elements with a concentration above 1.5 mg/g were quantified using a multielement standard for calibration. Data were expressed as mean  $\pm$  standard deviation. Student T-test were used to analyse the significance of the results. P values lower than 0.05 were considered as statistically significant.

#### 2.5 MIC calculation

To estimate the Minimum Inhibitory Concentration (MIC), the preparations were tested in serial dilutions 1:2 or 1:3 in a round-bottomed 96 well plate (Greiner Bio-One). 100  $\mu$ L of the medium used were first added to all wells. For a cascade dilution of 1:2, 100  $\mu$ L of the preparation to be tested (after a preliminary dilution) was added to well 1. After mixing, 100  $\mu$ L was removed and added to the following well. This step was repeated until well 10, after which 100  $\mu$ L was ejected. All wells except the sterility controls were then inoculated with 100  $\mu$ L of a bacterial overnight culture diluted to an optical density 600 nm (OD<sub>600</sub>) of 0.0002, for a final OD<sub>600</sub> of 0.0001 in each well. Plates were then agitated overnight (~18 hrs) at 37°C. Observation of MIC was made by eye and by reading of the OD<sub>600</sub> by an Infinite TECAN 200/M200 Pro. MIC was taken to be the lowest concentration at which bacterial growth was fully inhibited.

#### 2.6 Disk diffusion test

300  $\mu$ L of a bacterial overnight culture diluted to an OD<sub>600</sub> of 0.02 for LB medium, or 0.05 for SM medium were spread on a Petri dish poured with the same medium. After 10 minutes drying,

5-20  $\mu\text{L}$  of the samples to be tested was directly deposited on the dish. Dishes were finally left overnight (18 hrs) at  $37^{\circ}\text{C}$ , and pictures taken.

## 2.7 Cell viability

### 2.7.1 Cell incubation

RAW264.7 (TIB-71, ATCC) are monocytes/macrophages from mouse, widely used as model cell in inflammation studies. The incubation with the preparations (passed through a  $70\ \mu\text{m}$  filter, Flowmi Cell Strainer) was conducted over 3 days. The first day, 50,000 cells/well were seeded with filtered preparations, concentrations ranging from 0.016 to  $50\ \mu\text{L}/\text{mL}$ , to a final culture medium volume of  $100\ \mu\text{L}$  in 96 well plate flat bottom during 24 hrs at  $37^{\circ}\text{C}$ . On day 2, cells were stimulated with  $50\ \text{ng}/\text{mL}$  of lipopolysaccharides (LPS). On day 3, cells and supernatants were collected for subsequent analysis.

### 2.7.2 Evaluation of cell viability by MTS assays

24 h following treatment, mitochondria activity that is related to cell metabolic activity and thus cell viability was assessed using CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI). Briefly, after supernatant removal,  $100\ \mu\text{L}$  PBS 1X and  $20\ \mu\text{L}$  MTS solution were added per well. Cells were incubated at  $37^{\circ}\text{C}$  for 40 min and absorbance was measured at 490 and 700nm on a SAFAS spectrophotometer (SAFAS, Monaco). Cell viability was determined as the optical density ratio between sample and control cells and expressed as percent.

## 2.8 Inflammation evaluation

### 2.8.1 NO production

Concentrations of nitrogen dioxide in cell supernatants (representative of the nitric oxide produced by activated cells) were evaluated by Griess test. Briefly, 60  $\mu$ L of Griess reagent (v/v mixture of 58.1 mM p-aminobenzene sulfonamide in 30% acetic acid and 3.9 mM n-(1-naphthyl)ethylenediamine dihydrochloride in 60% acetic acid) were added to 40  $\mu$ L of supernatant and the absorbance at 543 nm was measured and compared to a sodium nitrite standard curve.

### 2.8.2 *TNF- $\alpha$ production*

To determine the effects of treatment on the production of inflammatory cytokines (*i.e.*, TNF- $\alpha$ ), cells were cultured and treated as described in the previous section. Supernatants collected from wells of a 96-well plate were assayed using specific enzyme-linked immunosorbent assay (ELISA). Purified anti-mouse TNF $\alpha$  antibody clone 1F3F3D4 and biotinylated anti-mouse TNF $\alpha$  antibody clone XT3/XT22 were purchased from eBiosciences/thermoFischer Scientific (Waltham, MA, USA). Avidin HRP was from Jackson (West Grove, PA, USA).

Briefly, capture antibody was diluted to 1  $\mu$ g/mL in a 0.05 M pH 9.6 carbonate/bicarbonate buffer and coated 1 night at 4°C before blocking with PBS 0.05% Tween 20 1% BSA (1h, 37°C). Samples were then diluted with PBS and incubated with capture antibody (2h, 37°C) before the biotinylated detection antibody diluted to 0.5  $\mu$ g/mL in PBS 0.05% Tween 20 1% BSA was added (1 h, 37°C). Avidin HRP was then introduced (45 min, 37°C) and revelation was conducted by adding a solution of 1.25 mM tetramethylbenzidine and 13.05 mM H<sub>2</sub>O<sub>2</sub> in 0.1 M pH 5 citrate buffer. Revelation was finally stopped by addition of 1 M HCl and absorbance was measured at 450 nm.

### 2.8.3 *Statistical analysis*

Data were expressed as mean  $\pm$  standard deviation. Mann-Whitney U-tests were used to analyse the significance of the results. P values lower than 0.05 were considered as statistically significant.

## Results

### 3.1 Global analysis of materia medica in the *aqrābādhīnat*

Arab scientists were really innovative, not only by the incorporation of new substances, simple or compound drugs, specific to the biotope of the places where they lived, but also in methodology, the establishment of professional practices with the separation of the pharmaceutical and medical professions. The growth of the medical field and the complexity of this profession also necessitated the codification of the pharmacy and the classification of the drugs, for a better implementation of the medical practice, and a more effective application of the treatments and development of medicines and remedies.

The medical material had three origins: vegetable (*nabāt*), animal (*min al-ḥayawn*) and mineral (*ma'ādin*) ; an analysis of the different sources showed that the distribution of substances varied only slightly (Table 1) in the *aqrābādhīn* as a function of time or geographical area with a percentage between 75,7 and 79,5% of the medical material which came from the vegetable kingdom, the rest being equally divided between animal origin or mineral origin.

### 3.2 Metals used in the *aqrābādhīn*

If the vegetal substances were mostly used, the sources revealed the systematic presence of simples drugs of mineral origin. We have identified 36 mineral products in the Arab pharmacopoeia (Table 2), showing that a wide variety of metals and minerals have been used for curative purposes, mainly minerals which were naturally available. We did not report clays which were also used in medicine back to antiquity [27], but which formulation is more complex. Most products were extracted from rocks in the native state. The minerals could be natural inorganic compounds but also chemical compounds extracted from deposits, produced by pyrolysis, or even transformed / synthesized. Indeed, the Arabs perfected the techniques of

chemistry, evaporation, melting metals, dissolution techniques in the nitric acid, sulfuric acid, hydrochloric acid and aqua regia. Thanks to these techniques, the Arab chemists performed various mineral combinations: silver nitrate, mercury oxide, copper sulphate, alum or hydrated iron. They also used crushed precious stones, garnet, hyacinth, topaz, emerald, sapphire, eagle stone. Some pills were gilded with precious metals, including gold that had several therapeutic virtues.

### 3.3 Analysis of the metals used in infection care

To analyse more in details the minerals used to treat symptoms that could be related to bacterial infection, we extracted the remedies specifying three types of symptoms: the symptoms affecting the respiratory system such as cough, chest pains (chest pain, lung ulcers, infection, mucus, etc.), medications useful in the treatment of skin and mucous membrane (abscesses, pustules, ulcers, scrofula, fistulas) as well as those intended to treat urinary tract infections, since all these affections can have a bacterial origin. We have obtained a total of 92 remedies, some of which treating two of the three symptoms. These remedies were distributed as: respiratory disorders (40), skin disorders (14), and urinary disorders (38).

Of the 40 respiratory drug remedies, only 3 are found in which mineral elements are used, and in these cases a clay (containing the metal) was introduced as a binder for galenic purposes. A single cough sputum preparation contained hematite mixed with plants, scorched and powdered mussel shells, coral and amber. Among the 38 preparations intended to cure the diseases of urinary infections, most of them -35- were ingested (infusions). On the three left, one contained mercury and another contained potash and lime. The first is applied as a clyster while the second is an enema. Altogether, except for clay or hematite, drugs containing mineral elements were not ingested, which argues in favour of a consideration of their potential toxicity. On the other hand, the treatment of skin disorders lead to a wide use of minerals since among the 14 remedies

listed, 10 contained at least one mineral element. The occurrence of metals in these remedies was reported in Table 3.

### 3.4 Selection of a remedy

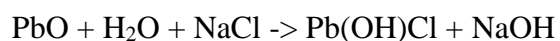
We then focused on remedies to cure skin disorders. Copper was the most frequently used metal (5 times), followed by litharge (2 times). We followed up a remedy containing mainly metals, among them litharge (PbO) and quicklime (CaO), described by Al-Kindī. As this remedy contained few organic ingredients, and no plants, we believed it could be easier to follow the transformations imposed to these metals. Even if nowadays, Pb is only considered through its toxicity, it has been widely used in past medicines. Studying different remedies based on Pb could help to evaluate the mastery of metal used by these physicians.

The remedy is described as followed: “ It draws out what is in it, cleanses it, cicatrizes the flesh. It is good and tested: it contains 1 part of quicklime and 1 part of litharge. Each is strained well through clay. Both are covered with the best wine vinegar until they are soaked in it. Olive oil is poured on them and also sheep fat in a measure that is enough for both. It is a measure of half of each of them. It is boiled together and stirred with a rod until it is mixed and it is almost black. It is left. The heating is renewed until the viscosity is like thick gathered honey. It is applied to the wound. It is tested and good with God’s help.”

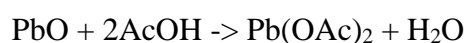
### 3.5 Metal transformation and formulation

Litharge-based compounds were widely used from the antiquity either in the formulation of make-up materials, or in remedies. We wondered whether its use in both types of application was comparable. A recent study on the chemical analysis of make up samples found in Egyptians tombs, confirmed by the reconstitution of ancient recipes, have shown that two non-natural Pb chlorides (laurionite  $\text{Pb}(\text{OH})\text{Cl}$  and phosgenite  $\text{Pb}_2\text{Cl}_2\text{CO}_3$ ) were purposely

synthesized, and triggered a pro-inflammatory activity [28]. In that case, the transformation of litharge (through a process already described by Discorides) was as follow:



The description of the ointment used by Al-Kindī to cure abscesses followed a different transformation process of litharge, due to the vinegar that contains acetic acid. This process was often used in the past to solubilize litharge in Pb acetate:



It should be noted that quicklime underwent the same transformation ( $\text{CaO} + 2\text{AcOH} \rightarrow \text{Ca(OAc)}_2 + \text{H}_2\text{O}$ ).

Thus depending on final application, litharge could be submitted to different transformation processes. We wondered whether this differences conferred also different activities to the metals.

### 3.6 Reproduction of the remedy

To reproduce as faithfully as possible the remedy 1 g of quicklime (CaO) and 1 g of litharge (PbO) were loaded on a celite 545 column to reproduce the step of filtration on clay. Vinegar was then added to the column until 50 mL of filtrate was obtained. We added the same volume of olive oil as well as 4 g of sheep fat after the filtration, and heated the preparations to 150°C, but we only got two separated phases. We obtained the viscous black ointment described when the preparation was passed to the flame (higher temperature). Figure 2 summerizes the preparation steps and give an idea of the intermediate/final products.

To test the role of the different compounds, we prepared different versions of the remedy (named preparations), omitting sometimes one ingredient, or step... (Figure 3).

The vinegar was essential to dissolve the litharge, as well as the quicklime in their acetate salt form. It should be noted that the filtration through celite (or any clays) that contained different salts could enable the formation of different lead or calcium salts, by exchange between acetate and other counter-ions. The final formulary contained thus lead and calcium salts, vinegar, olive oil and sheep fat. The efficacy of metal solubilisation by vinegar was assessed by analyzing the metal content through ICP-AES. Pb was at a concentration of 10 mM (+/- 15 %) in the solution recovered after filtration (50 ml recovered), yielding 10% of the initial litharge deposited on the clay. Ca was better solubilized, as almost 50 mM (+/- 15 %) were recovered. The quicklime enabled to buffer the preparation, increasing the pH from 3-4 to 6. The clay was used to filtrate the product, so that litharge / quicklime were prevented from ending up in the final product. The olive oil, sheep fat and heating step were indispensable to get the black ointment consistency described by Al-Kindī. Omitting one of these ingredient/step did not allow the obtention of the ointment. They thus have a galenic function. Since the ointment was not soluble, and thus its activity difficult to test, we only analysed the biological activity of preparations without olive oil and sheep fat.

### 3.7 Trace metal content

We further measured the concentration of several metals (biologic or toxic) and analysed their variation among some preparations (preparations containing either litharge (3), quicklime (4), both (5) or neither ingredient (2) (Figure 5) by ACP-AES. While as expected, Ca was more concentrated in preparations 4 and 5, and Pb more concentrated in preparations 3 and 5, some other metals (either toxic like Al or non toxic as K, Mg, Na, Zn) have been significantly found in higher concentrations in the preparation 3 than in the others, according to Student T-test statistical analysis. This may be explained by the acidity of preparation 3 leading to a better



solubilization of different metals present in the celite, and thus higher concentrations in the eluate.

### 3.8 Antibacterial activity

The antibacterial activity of the preparation obtained was tested on three different pathogenic bacteria, belonging to the ESKAPE group of alarming pathogens for their resistance to antibiotics: the gram positive *Staphylococcus aureus*, the gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. Two methods were used to assess the antibacterial activity, based on solid or liquid LB media. Different preparations were tested, which were prepared either without quicklime or litharge (Figure 3) to study their antibacterial effect. We further performed control experiments only with vinegar, to dissociate the role of the Pb or Ca salts (obtained after filtration on clay with vinegar in preparations 3 and 4 respectively), to that of vinegar alone (Figure 3). Two control preparations of vinegar were used, either vinegar passed through clay (control 1) as a control for the acidic preparation 3 (pH 3-4), or buffered vinegar passed through clay (control 2) as a control for the preparation 4 or 5 (pH 6). As a positive control, we also exposed the bacteria to 10 µl of tobramycin (0.1 µg/mL; Figure 4, positive control). We loaded 10 µL of preparations on plates (Figure 4). Beside the central plot, which corresponds to some precipitation of the preparations once deposited on the plate (metals are usually only partly soluble in physiological conditions), a hole in the bacterial plate reflected the antibacterial activity of the preparation. When comparing the preparation 3 (Figure 4, spots 3) to that of the vinegar control 1 (Figure 4, spots 1), both carried a similar antibacterial activity, meaning that Pb salts contained in preparation 3 had probably no antibacterial activity by itself. The preparation 4 (Figure 4, spots 4), however, showed an antibacterial activity when compared to that of the buffered vinegar control 2 (Figure 4, spots 2), showing that the Ca salts contained in preparation 4 carry an antibacterial activity. The preparation 5, containing both Pb and Ca salts,

(Figure 4, spots 5) did not greatly improve the effect on bacteria as compared to that of preparation 4 (Figure 4, spots 4). Thus, the antibacterial activity of the remedy, as far as we can test, relied most probably on Ca salts.

Since the different version of the remedy partly precipitated on the plates, it was important to test these fractions in liquid, at lower initial concentration, that could minor this adverse effect. We then tested the antibacterial properties of the preparations in liquid (in 96-well plates), by following the growth of the bacteria in the presence of serial dilution of the different preparations. As long as the concentration of the preparations were lethal for the bacteria, they did not grow. The concentration used in the last well in which the bacteria did not grow gave the Minimum Inhibitory Concentration (MIC, Table 4). When the dilution was more important, the preparations were not able to inhibit bacterial growth anymore. The MIC of the preparation 3 was pretty similar to that of vinegar control 1, for the three bacteria, that confirmed the weak antibacterial activity of Pb salts. The preparations 4 containing Ca salts, on the opposite, triggered an antibacterial activity (MIC = 15  $\mu\text{L}/\text{mL}$ , *e.i.* 15 $\mu\text{L}$  of preparation diluted in 1 mL of medium), which was absent when buffered vinegar control 2 was tested. As for the plate, the preparation 5 containing Pb and Ca salts did almost not improve the activity of preparation 4. Altogether, whether tested on plate or in liquid, the remedies gave similar results; the absence of bactericidal effect of lead in this remedy might probably not be due to precipitation in LB medium. Since LB is a very rich medium, not reflecting the one on the skin which might be poorer, we also tested the activity of the preparations in succinate medium, a poorer medium. Indeed, Pb ions target many biomolecules that are apt to interact with the most common divalent cations [29]. There is thus a competition between Pb and biological metals for specific function (ion channels, enzymes, and proteins). The use of this poor medium (including poor metal concentrations, at least 100 fold lower [30]) will change the ratio between Pb and the other metals, and thus potentially increase its toxicity. However, when we performed these tests on

*P. aeruginosa*, we observed similar results than in LB. Thus, changing the composition of the medium, or the ratio between toxic metals/biological metals might not change drastically the bactericidal activity of either Pb or Ca salts.

Altogether, the preparation tested enabled to show that the remedy promoted an antibacterial activity, mainly carried by Ca salts. This activity was however low, but it should be noted that these metal preparations partly precipitated in the media, so that the real bactericidal activity of the preparations might be higher than the observed ones. Further, the ointment was directly applied to the infection site according to Al-Kindī, which might enable to reach these high concentrations precisely at this infected site. Interestingly also, while plant-based remedies often show enhanced activity against gram positive versus gram negative bacteria, these metal preparations were similarly active against both type of bacteria.

### 3. 9 Anti-inflammation properties

During oedema development following bacterial infection, immune response, especially inflammation, is an important part of the symptoma. To evaluate the possible effect of the preparations on inflammation, we used a very simple cellular model to partially mimick *in vitro* the inflammatory response : activated mouse macrophage cell line, RAW264.7. We tested the preparations 4 or 5, but not the preparation 3, as acidic pH is detrimental for RAW264.7 cells. We showed the results for two lots of preparations 5 (Figure 6).

#### 3.9.1 Toxicity on RAW cells

First, we investigated the toxicity of the preparations, using measurement of mitochondrial metabolism that was proportional to cell viability. These preparations were added at various concentration to RAW cell culture. As shown on Figure 6A, preparation 4 (without Pb salts) had no effect on cell viability until of 50  $\mu\text{L}/\text{mL}$ . By contrast, in the presence of Pb salts, the two distinct preparations 5 induced cell cytotoxicity for concentrations above 0.1  $\mu\text{L}/\text{mL}$ . In the

rest of the experiments, we used a concentration of 0.08  $\mu\text{L}/\text{mL}$  at which the preparations does not affect cell viability.

### 3.9.2 Production of NO and TNF- $\alpha$

The pro-inflammatory or anti-inflammatory activities of the preparations were then estimated by assessing the amount of various inflammation mediators produced by the activated RAW cells such as nitric oxide (NO) (Figure 6A) or pro-inflammatory cytokines such as TNF- $\alpha$  (Figure 6B). As a control, the production of NO and TNF- $\alpha$  was measured when no preparations were added to the RAW cells.

As shown in Figure 6, after 24h of contact with any preparation, no observable pro-inflammatory activity could be detected, as of NO and TNF- $\alpha$  were produced (label '0' in abscisses of Figure 6B and C).

To evaluate the potential anti-inflammatory activity of the preparations, RAW cells were activated by lipopolysaccharide (label 'LPS' in abscisses of Figure 6B and C), a chemical compound that mimicks infection and induces inflammation, after treatment with the preparations during 24 hrs. As expected, LPS induced the production of NO (Figure 6B, control) and TNF- $\alpha$  (Figure 6C, control). The preparations 5 containing Pb salts, induced a decrease of NO and, in a lesser extend, of TNF- $\alpha$  whereas the preparation 4 without Pb salts had no effect. Altogether, these results showed that preparations containing Pb salts had anti-inflammatory properties.

### **3. Discussion**

Due to antibiotic overuse during the last decades, bacteria have developed resistance in such a way as to represent an important health risk for the next years. Unfortunately, the antibiotic discovery pipeline has been reduced in the same period and we currently lack new lead compounds. There is thus an urgent need for new molecules/strategies to fight these resistant bacteria. Among the possibilities, plant-derived compounds and other natural substances like minerals may represent a rich source of products that could inhibit or mitigate current antibiotic-resistant pathogens. In the last years, research on nature-derived drugs has increasingly turned to historical medical texts, both to study the development of pharmacopoeias and to identify candidate species for the discovery of new drugs [31], [32]. Medieval societies used these natural substances to treat diseases clearly identifiable by the trained eye as microbial infections. Specifically, Arab physicians have produced a developed pharmacopoeia containing thousands of preparations that could be re-examined in the light of current scientific research techniques. We focused our study on metal-plant derived remedies, as they were often prescribed when plant-only remedies were not efficient enough. Even if promising, such remedies deserved only few studies, due to a reluctance toward metals as compared to plants, difficulties to identify the past formulation of metals, and to understand their mode of action. Thus, to promote again such studies, it is essential to bring a proof of concept that such remedies are worth studying, through examination of the potential brought by metals.

We based our study on historical text analyses from three different, well recognized Arab scientists from the 9-12th century. Even if plants, as we could expect, were extensively used, ingredients from mineral origin still represented 10% of all, with no less than 36 different minerals. When it came to remedies against infections that could be applied externally, as for skin infection, the use of minerals grew to 70%. We then focused our study on a simple remedy,

prescribed by Al-Kindī to cure abscesses and containing mainly metals, to bring a proof of concept that it is possible to analyse their formulation, to assess the mastery of ingredient transformation by the physicians, their awareness about toxicity, and finally the potentialities brought by the use of metals.

The remedy studied here contained litharge, quicklime, vinegar, olive oil and sheep fat. While olive oil and sheep fat had mainly a galenic role, we studied the function of the other ingredients. Vinegar allowed the solubilization of litharge and quicklime. For litharge and quicklime, we turned to the historic indications to study their role. The remedy was prescribed as such: “ It draws out what is in it, cleanses it, cicatrizes the flesh”. Thus, the remedy could be expected to carry different actions, not only a bactericidal one, but also an pro or anti-inflammatory one. Wound healing usually consists of two phases [33], [34], one pro-inflammatory, to combat the infection and destroy the infectious agents (named acute inflammation) , and one anti-inflammatory to enable a repair of the wound (named inflammation resolution). This latter stage is necessary for re-epithelialisation, re-vascularisation, and fibroblast regeneration [35], [36]. In an *in vitro* model of inflammation, we observed that two inflammation markers (NO and TNF) was not increased by our remedy. On the contrary, the remedy decreased the production of these two markers induced by a molecule mimicking infection (LPS) suggesting an anti-inflammatory effect of the remedy. Moreover, this effect was not observed in presence of litharge showing the role of the Pb in this effect. All together, our data showed that the remedy mimicks the two phases of inflammation, the Ca salts contained in the remedy triggering the bactericidal activity, as it is the case for the first pro-inflammatory step, while the Pb salts promoted the subsequent anti-inflammatory, cicatrisation phase.

One difficulty when working on preparations with non-pure materials (as it is the case here with quicklime, celite, or vinegar) is the potential contamination of the final product with undesired ingredients, like other metals that could be responsible of some of the observed effects, or

induce other side effects. This has been particularly depicted for some herbal preparations [17], in which Pb contamination was involved in toxic side effects. In our preparations, we indeed found different metals that originated probably from the celite (as any other clay, celite contained trace metals elements). The fact that higher concentrations of most metals were found in the preparation 3 (preparation without quicklime), conferred another role to quicklime, as buffering the vinegar avoided a higher solubilisation of these metals. In this study, the experimental protocols (including the controls) enabled to avoid some bias in the conclusions. Indeed, the anti-inflammatory properties have been deduced from comparison of preparations containing or not litharge, which purity is assessed  $> 99.99\%$  by the manufacturer. The antibacterial effect of preparation 4 (with quicklime) could in that case be due to metals contained in the commercial quicklime, but only the calcium has been shown in higher concentration in preparation 4 as compared to the others. These results nevertheless pointed out the importance of checking the presence of trace elements, especially for preparations containing non-pure ingredients.

The direct application of the remedy on the infection site could have two beneficial effects: it might enable to reach a high concentration of the ingredients at this specific site, promoting the antibacterial and the anti-inflammatory effects, while the diffusion of the ingredients in the body through the skin might remain low, and thus their toxic effect mainly restricted to the infected area. Altogether, with a very simple recipe, these past physicians designed a complete and synergistic remedy to combat abscesses, while restricting the toxic effect of litharge to the site of infection.

It is further a first example showing that different metal manufacture were evolved to improve the therapeutic potentials of metals. A previous study showed that litharge could be purposely transformed in laurionite and phosgenite, with pro-inflammatory properties which were added to make-up to prevent infection [28]. Our results showed that through a different process of

transformation, the physicians could obtain soluble Pb with anti-inflammatory properties, that promoted cicatrization. These formulations were not purposely prepared to trigger pro/anti-inflammation, as these physicians did not have the molecular knowledge for that. How did they come to such unattended medical conclusions is still appealing and intriguing. Yet, they knew that these formulations were adapted to preventive care, or skin infection cure, through careful experimental observations, and used these different preparations purposely.

Pb has proven highly toxic for human [39], [29], which contrasts with its wide use in the past especially as anti-infective (either in make-up to promote a protective immunity or in remedies themselves), that still remains questionable. Nevertheless, Pb toxicity was known even in Antiquity. Its therapeutic use was documented and framed as reported by Lanoë [40]. As anti-infective, Pb was mainly used externally, which limits the diffusion of this toxic metal in the body, while concentrates its action on the infection site. Knowledge about metal toxicity was not limited to Pb. For example, Hg (mercury) formulations were also used [41]. Its toxicity was also well-known since ancient time, as reported by Pliny the Elder [13], and past chemists knew that different Hg formulations triggered different toxicity. Hg was thus preferentially used as cinnabar (sulphur salt, HgS), which toxicity has been recently estimated to be  $> 3$  log lower than current well-known and toxic mercury salts (Me-Hg, HgCl<sub>2</sub>) [42]. Thus, it is possible that these ancient and careful precautions changed the balance advantage/risk on the use of such metals. Altogether, it appears that the past physicians were aware of metal toxicity, and that their use could be carefully framed.



#### **4. Conclusion**

Of course we do not wish to promote the re-use of Pb in medicine, but our data illustrated the capacity of these physicians to master the transformation and formulation of metals, while handling the toxicity of these hazardous materials. Being aware of these past knowledges, mastering, risk prevention in metal use, should help considering again these ancient remedies for more than shamanism or charlatanism. The knowledges acquired by these physicians deserve more attention, and it is likely that research on these types of ancient remedies combining plant and metal, maybe targeted on less toxic metals, could still reveal unexpected features, original organo-metallic compounds or therapeutic synergy. We are also aware that promoting again the use of such formulations will also require consideration of the various trace elements that these formulations may contain (due to contamination/lack of purity...), and their beneficial/detrimental effects on the final product.

#### **Author contributions**

V.P., P.F. conceived this study and drafted the manuscript. B.A., E.A., C.S. performed the experiments. P.F., V.P., and S.F. analysed the data. C.V., L.C., H.A.B, L.S. formulated hypotheses, and contributed to manuscript preparation, G.L.A.M., S.F., I.J.S. participated to the experimental design of the study. All authors reviewed and approved the manuscript.

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## References

- [1] K.J. Waldron, N.J. Robinson, How do bacterial cells ensure that metalloproteins get the correct metal?, *Nat. Rev. Microbiol.* 7 (2009) 25–35. <https://doi.org/10.1038/nrmicro2057>.
- [2] C. Andreini, I. Bertini, A. Rosato, A hint to search for metalloproteins in gene banks, *Bioinforma. Oxf. Engl.* 20 (2004) 1373–1380. <https://doi.org/10.1093/bioinformatics/bth095>.
- [3] T. Bardinet, *Les Papyrus médicaux de l’Égypte pharaonique. Trad. intégrale et comment*, Fayard, Paris, 1995.
- [4] J. Campbell, Pharaohs and the first prescriptions, *Pharm. J.* (2007) 735–737.
- [5] Credé, Die Verhütung der Augenentzündung der Neugeborenen, *Arch. Für Gynäkol.* 17 (1881) 50–53. <https://doi.org/10.1007/BF01977793>.
- [6] J.M. Sims, On the treatment of vesico-vaginal fistula. 1852, *Int. Urogynecol. J. Pelvic Floor Dysfunct.* 9 (1998) 236–248. <https://doi.org/10.1007/BF01901610>.
- [7] J. Pereira, T.B. Crosby, G.O. Rees, A.S. Taylor, K.C. London, S.T. Hospital, *The elements of materia medica and therapeutics, Third edition enlarged improved, including notices of most of the medicinal substances in use in the civilized world, and forming an encyclopaedia of materia medica.*, Printed for Longman, Brown, Green and Longmans, London :, 1849. <https://doi.org/10.5962/bhl.title.109750>.
- [8] N.D.C. Hodges, N.D.C., The Value of Mercuric Chloride as a Disinfectant, *Science.* 13 (1889) 62–64.
- [9] P. Ehrlich, A. Bertheim, Über das salzsaure 3.3’-Diamino-4.4’-dioxy-arsenobenzol und seine nächsten Verwandten, *Berichte Dtsch. Chem. Ges.* (1912) 756–766.
- [10] E.L. KEYES Jr., THE TREATMENT OF GONORRHEA OF THE MALE URETHRA, *J. Am. Med. Assoc.* 75 (1920) 1325–1329. <https://doi.org/10.1001/jama.1920.02620460023008>.
- [11] A.D. Frazer, TELLURIUM IN THE TREATMENT OF SYPHILIS., *The Lancet.* 216 (1930) 133–134. [https://doi.org/10.1016/S0140-6736\(01\)09037-7](https://doi.org/10.1016/S0140-6736(01)09037-7).
- [12] M. Wellman, University of Glasgow. Library, *Pedanii Dioscuridis De materia medica libri quinque* [electronic resource], Berolini : Weidmann, 1906. [http://archive.org/details/b21459162\\_0001](http://archive.org/details/b21459162_0001) (accessed November 5, 2020).
- [13] H. Le Bonniec, *Histoire naturelle. Livre XXXIV - Pline l’Ancien*, Paris, 2002. <https://www.lesbelleslettres.com/livre/867-histoire-naturelle-livre-xxxiv> (accessed November 5, 2020).
- [14] (Christian) BONAHE, L’affaire du Stalino : et ses conséquences réglementaires, 1954-1959 “Sécurité sanitaire” et innovation thérapeutique en France il y a 50 ans., *Aff. Stalino Ses Conséq. Réglém. 1954-1959 Sécurité Sanit. Innov. Thérapeutique En Fr. Il 50 Ans.* 57 (2007) 1501–1505.
- [15] J. Parascandola, From Mercury to Miracle Drugs: Syphilis Therapy over the Centuries, *Pharm. Hist.* 51 (2009) 14–23.
- [16] M.A. Riva, A. Lafranconi, M.I. D’Orso, G. Cesana, Lead Poisoning: Historical Aspects of a Paradigmatic “Occupational and Environmental Disease,” *Saf. Health Work.* 3 (2012) 11–16. <https://doi.org/10.5491/SHAW.2012.3.1.11>.
- [17] M.A. Mikulski, M.D. Wichman, D.L. Simmons, A.N. Pham, V. Clotey, L.J. Fuortes, Toxic metals in ayurvedic preparations from a public health lead poisoning cluster investigation, *Int. J. Occup. Environ. Health.* 23 (2017) 187–192. <https://doi.org/10.1080/10773525.2018.1447880>.
- [18] J.A. Lemire, J.J. Harrison, R.J. Turner, Antimicrobial activity of metals: mechanisms, molecular targets and applications, *Nat. Rev. Microbiol.* 11 (2013) 371–384. <https://doi.org/10.1038/nrmicro3028>.

- [19] G.B. and J. Gabbay, Copper, An Ancient Remedy Returning to Fight Microbial, Fungal and Viral Infections, *Curr. Chem. Biol.* 3 (2009) 272–278. <https://www.eurekaselect.com/93409/article> (accessed October 26, 2020).
- [20] E. Connelly, C.I. Del Genio, F. Harrison, Data Mining a Medieval Medical Text Reveals Patterns in Ingredient Choice That Reflect Biological Activity against Infectious Agents, *MBio.* 11 (2020). <https://doi.org/10.1128/mBio.03136-19>.
- [21] A.E. Al-Snafi, Therapeutic properties of the medicinal plants: a review of their antibacterial activity (Part 1), *Int. J. Pharm. Ther.* 6 (2015) 137–158.
- [22] F. Harrison, A.E.L. Roberts, R. Gabriłska, K.P. Rumbaugh, C. Lee, S.P. Diggle, A 1,000-Year-Old Antimicrobial Remedy with Antistaphylococcal Activity, *MBio.* 6 (2015) e01129-15. <https://doi.org/10.1128/mBio.01129-15>.
- [23] O. Kahl, *The Small Dispensatory, Sahl Sābūr Ibn*, BRILL, leiden, 2003.
- [24] O. Kahl, *Sābūr ibn Sahl's Dispensatory in the Recension of the 'Aḡudī Hospital.*, Brill, Leiden, 2009. [https://www.academia.edu/4245202/S%C4%81b%C5%ABr\\_ibn\\_Sahls\\_Dispensatory\\_in\\_the\\_Recension\\_of\\_the\\_%CA%BFA%E1%B8%8Dud%C4%AB\\_Hospital\\_Islamic\\_Philosophy\\_Theology\\_and\\_Science\\_78\\_By\\_Oliver\\_Kahl\\_pp\\_1x\\_265\\_Leiden\\_and\\_Boston\\_Brill\\_2009](https://www.academia.edu/4245202/S%C4%81b%C5%ABr_ibn_Sahls_Dispensatory_in_the_Recension_of_the_%CA%BFA%E1%B8%8Dud%C4%AB_Hospital_Islamic_Philosophy_Theology_and_Science_78_By_Oliver_Kahl_pp_1x_265_Leiden_and_Boston_Brill_2009) (accessed November 5, 2020).
- [25] M. Levey, *The medical formulary, or Aqrābādihīn of Al-Kindi*, University of Wisconsin Press, Madison, 1966.
- [26] O. Kahl, *The Dispensatory of Ibn At Tilmid: Arabic Text, English Translation, Study and Glossaries, Bilingual édition*, Brill, Leiden ; Boston, 2007.
- [27] E. Photos-Jones, C. Keane, A.X. Jones, M. Stamatakis, P. Robertson, A.J. Hall, A. Leanord, Testing Dioscorides' medicinal clays for their antibacterial properties: the case of Samian Earth, *J. Archaeol. Sci.* 57 (2015) 257–267. <https://doi.org/10.1016/j.jas.2015.01.020>.
- [28] I. Tapsoba, S. Arbault, P. Walter, C. Amatore, Finding Out Egyptian Gods' Secret Using Analytical Chemistry: Biomedical Properties of Egyptian Black Makeup Revealed by Amperometry at Single Cells, *Anal. Chem.* 82 (2010) 457–460. <https://doi.org/10.1021/ac902348g>.
- [29] P. Mitra, S. Sharma, P. Purohit, P. Sharma, Clinical and molecular aspects of lead toxicity: An update, *Crit. Rev. Clin. Lab. Sci.* 54 (2017) 506–528. <https://doi.org/10.1080/10408363.2017.1408562>.
- [30] O. Cunrath, V.A. Geoffroy, I.J. Schalk, Metallome of *Pseudomonas aeruginosa*: a role for siderophores, *Environ. Microbiol.* 18 (2016) 3258–3267. <https://doi.org/10.1111/1462-2920.12971>.
- [31] M. Dal Cero, R. Saller, C.S. Weckerle, The use of the local flora in Switzerland: A comparison of past and recent medicinal plant knowledge, *J. Ethnopharmacol.* 151 (2014) 253–264. <https://doi.org/10.1016/j.jep.2013.10.035>.
- [32] P.O. Staub, L. Casu, M. Leonti, Back to the roots: A quantitative survey of herbal drugs in Dioscorides' *De Materia Medica* (ex Matthioli, 1568), *Phytomedicine.* 23 (2016) 1043–1052. <https://doi.org/10.1016/j.phymed.2016.06.016>.
- [33] M. Hesketh, K.B. Sahin, Z.E. West, R.Z. Murray, Macrophage Phenotypes Regulate Scar Formation and Chronic Wound Healing, *Int. J. Mol. Sci.* 18 (2017). <https://doi.org/10.3390/ijms18071545>.
- [34] A.C. Labonte, A.-C. Tosello-Trampont, Y.S. Hahn, The Role of Macrophage Polarization in Infectious and Inflammatory Diseases, *Mol. Cells.* 37 (2014) 275–285. <https://doi.org/10.14348/molcells.2014.2374>.
- [35] B. Shook, E. Xiao, Y. Kumamoto, A. Iwasaki, V. Horsley, CD301b+ Macrophages Are Essential for Effective Skin Wound Healing, *J. Invest. Dermatol.* 136 (2016) 1885–1891. <https://doi.org/10.1016/j.jid.2016.05.107>.
- [36] J.M. Daley, S.K. Brancato, A.A. Thomay, J.S. Reichner, J.E. Albina, The phenotype of murine wound macrophages, *J. Leukoc. Biol.* 87 (2010) 59–67. <https://doi.org/10.1189/jlb.0409236>.

- [37] E. Shardlow, M. Mold, C. Exley, Unraveling the enigma: elucidating the relationship between the physicochemical properties of aluminium-based adjuvants and their immunological mechanisms of action, *Allergy Asthma Clin. Immunol.* 14 (2018) 80. <https://doi.org/10.1186/s13223-018-0305-2>.
- [38] E. Oleszycka, E.C. Lavelle, Immunomodulatory properties of the vaccine adjuvant alum, *Curr. Opin. Immunol.* 28 (2014) 1–5. <https://doi.org/10.1016/j.coi.2013.12.007>.
- [39] I.D. de Souza, A.S. de Andrade, R.J.S. Dalmolin, Lead-interacting proteins and their implication in lead poisoning, *Crit. Rev. Toxicol.* 48 (2018) 375–386. <https://doi.org/10.1080/10408444.2018.1429387>.
- [40] C. Lanoë, La céruse dans la fabrication des cosmétiques sous l’Ancien Régime (XVIe-XVIIIe siècles), *Tech. Cult. Rev. Semest. D’anthropologie Tech.* (2002). <https://doi.org/10.4000/tc.224>.
- [41] V.S. Vaidya, H.M. Mehendale, Mercuric Chloride (HgCl<sub>2</sub>), in: P. Wexler (Ed.), *Encycl. Toxicol.* Third Ed., Academic Press, Oxford, 2014: pp. 203–206. <https://doi.org/10.1016/B978-0-12-386454-3.00330-4>.
- [42] Q. Wu, Y.-F. Lu, J.-Z. Shi, S.-X. Liang, J.-S. Shi, J. Liu, Chemical form of metals in traditional medicines underlines potential toxicity in cell cultures, *J. Ethnopharmacol.* 134 (2011) 839–843. <https://doi.org/10.1016/j.jep.2011.01.031>.

## Figure Legends

**Figure 1. Text from Qanūn, Ibn Sīnā, Folio 10v, BNF, Ms Ar. 6454**

**Figure 2. Preparation of the remedy.** The photos illustrate the steps to prepare the remedy, as well as its aspect. For this experiment, 1 g of quicklime and 1 g of litharge were loaded on a syringe plugged with 10 g of celite 454 (**A**). Vinegar was then poured onto the syringe to obtain 50 mL of filtrate (**B**). The filtrate was recovered in a round-bottom flask, and 50 ml of olive oil as well as 4 g of sheep fat were added. The preparation was heated first at 150°C then passed to the flame (**C**) to obtain the final black ointment (**D**).

**Figure 3. Summary of the preparations tested.** All preparations were filtrated through clay with wine vinegar. Control preparations (in green) were prepared only with vinegar, that was buffered before the filtration step or not. Different other preparations were studied (in yellow), for which only litharge was passed through clay with vinegar (preparation 3), only quicklime (preparation 4) or both minerals (preparation 5). The importance of olive oil, sheep fat or heating step for galenic purposes (pink) was also assessed through preparations lacking one of these ingredient/step. The numbers refer to preparations that will serve to study the biological activities. The details of the preparations are depicted in 2.3 and 3.6. When required, statistical tests were used to assess the results and significance of the comparison (see section 2.4, 2.8.3).

**Figure 4. Antibacterial effect of the remedy preparations.** 300 µL of a bacterial (*S. aureus*, *E. coli* and *P. aeruginosa*) overnight culture diluted to an OD<sub>600nm</sub> of 0.02 were spread on LB plates. 10 µL of preparations were then dropped onto the plates which were left overnight (18 hrs) at 37°C, before pictures were taken. Each drop related to a specific preparation, which

number referred to those in Figure 3. The halo around the drops reflected the antibacterial activity of the preparations. Positive controls referring to tobramycin bactericidal activity on these bacteria have been added.

**Figure 5. Metal content of the preparations.** The presence of several metals have been monitored in the preparations 3, 4, 5 and in the control preparation 2. Samples were mineralized in closed vessels with nitric acid for trace analysis at 100°C for 1h. The detection of 30 elements in the clear obtained solution was realized at the proper dilution by ICP-AES (Varian 720ES). The concentration is given in mg/g of the dried preparations. Elements with a concentration above 1.5 mg/g were quantified using a multielement standard for calibration. The concentration of Mn in the control preparation 2 was below 1.5mg/g, and is thus not shown. P values were determined by Student's *t*-test (\* P<0,05, \*\* P<0,005).

**Figure 6. Cytotoxic effect and anti-inflammatory response of the preparations on RAW264.7 cells.** RAW264.7 cells were incubated with different concentrations of the preparations 4 and 5 (referring to Figure 3). Two different preparations 5 have been used (blue and red), to monitor intra-variation that could originate from the preparation protocol. Cytotoxicity was assessed using mitochondrial activity after 48 hours of contact (**6A**). As control, the cytotoxicity was assessed either without exposition of RAW cells to the preparations (purple), or with 5% DMSO (a toxic concentration, orange) and represented as a single point on the graph. (**6A**). The results are expressed as the percentage of breathing mitochondria corresponding to alive cells from 3 independent experiments. To study the inflammatory part (**6B** and **C**), RAW cells were exposed to the preparations for 24 hours (value '0' in abscisses), or further challenged with 50 ng/mL of lipopolysaccharide (value 'LPS' in abscisses) for 24 hours or not (value '0' in abscisses) (**6B** and **C**). Supernatants were then

collected. NO production was assessed by Griess reaction (n=3) and TNF $\alpha$  by ELISA (n=3). They reflect an activation of the macrophages, and thus a pro-inflammatory effect. Figure **6B** and **6C** represent respectively NO and TNF $\alpha$  concentrations observed in supernatant of cells cultured in presence of 0.08  $\mu$ L/mL of filtered remedies where no cell toxicity has been observed (**6A**). P values were determined by two-tailed Mann-Whitney U-test (\* P<0,05, \*\* P<0,005).



**Table 1:** Comparison of the nature of the medical matter

Source	Number of substances	% vegetal (%)	% animal (%)	% mineral (%)
Al-Kindī	311	79,5	9,6	10,9
Ibn Sahl	378	79,1	10,6	10,3
Ibn al-Tilmīdh	433	75,7	11,3	13,0

**Tableau 2:** Mineral medicinal substances and chemical formulas (X present, - absent)

Mineral	Formula	Sabur Ibn Sahl	al-Kindī	Al-Tilmidh
Silver	Ag	X	X	X
Realgar	As <sub>4</sub> S <sub>4</sub>	X	X	X
Orpiment	As <sub>2</sub> S <sub>3</sub>	X	X	X
Gold	Au	X	X	X
Quicklime	CaO	X	X	X
Gypsum	CaSO <sub>4</sub> , 2(H <sub>2</sub> O)	X	-	-
Cadmium	Cd	X	-	X
Copper	Cu	X	-	X
Burnt copper	Cu <sub>2</sub> O	X	X	X
Verdigris	Cu(OH) <sub>2</sub> , CuCO <sub>3</sub>	X	X	X
Rust	Fe <sub>2</sub> O <sub>3</sub> , 3 H <sub>2</sub> O	X	X	X
Haematite	α-Fe <sub>2</sub> O <sub>3</sub>	X	X	X
Pyrite	FeS <sub>2</sub>	X	X	X
Marcasite <sup>a</sup>	FeS <sub>2</sub>	-	X	-

Mercury	Hg	-	X	-
Cinnabar (or vermilion)	HgS	X	-	-
Vitriol <sup>b</sup>	-	X	X	X
Potash	KOH	X	X	X
Alun	KAl(SO <sub>4</sub> ) <sub>2</sub> , 12 H <sub>2</sub> O	X	X	X
Ammonium salt	NH <sub>4</sub> Cl	X	X	X
Borax	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 10 H <sub>2</sub> O	X	X	X
Litharge	PbO	X	X	X
White lead (ceruse)	PbCO <sub>3</sub> , Pb(OH) <sub>2</sub>	X	X	X
Red lead (minium)	PbO <sub>2</sub>	X	X	X
Lead sulfide	PbS	X	-	-
Sulphur	S	X	X	X
Antimony sulphide	Sb <sub>2</sub> S <sub>3</sub>	X	X	X
Scale (Calamine)	ZnCO <sub>3</sub>	X	X	-
Alkali <sup>c</sup>	-	-	X	X
Natron	Na <sub>2</sub> CO <sub>3</sub> ·10H <sub>2</sub> O	-	X	X

Tutty	ZnO	X	X	-
Ruby		-	X	-
Salt	NaCl	X	X	X
Salpeter	KNO <sub>2</sub>	-	X	-
Lapis Lazuli		-	-	X

<sup>a</sup>Another crystalline form of pyrite, slightly lighter in colour.

<sup>b</sup>The term *vitriol* (*qualqa* in Arabic) is not equivalent to the current term for concentrated sulfuric acid but refers to various sulphates according to the color that is affixed to the name. Thus, here is the blue vitriol [copper sulphate], the green vitriol [iron sulphate and basic copper sulphate], the yellow and red vitriol [basic iron sulphate] and finally the white vitriol [zinc sulphate; alumina sulfate, or even alum].

<sup>c</sup>It may be potash or ammonia, but it is rather a mixture of sodium carbonate and potassium obtained by slow combustion of certain plants.

**Table 3:** Number of occurrences of minerals present in the treatment of skin conditions

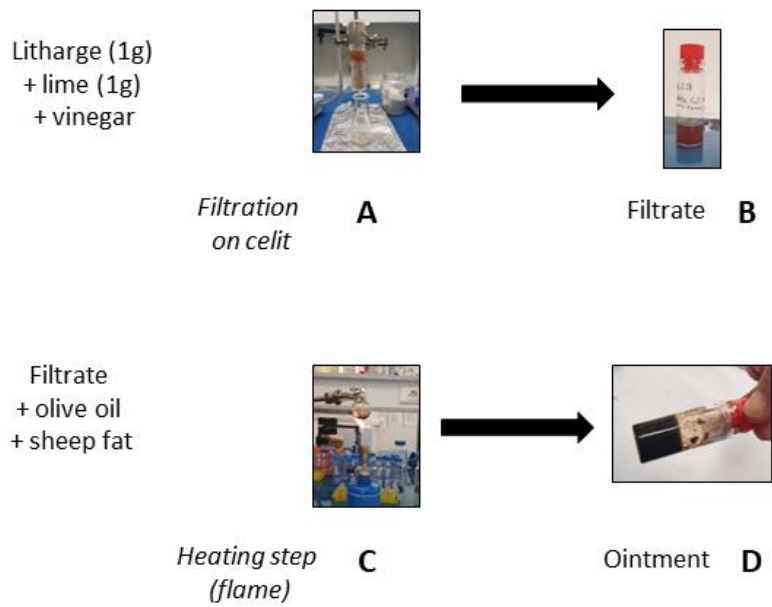
Mineral	Occurrences
Gold (Au)	1
Silver (Ag)	1
Litharge (PbO)	2
Minium (PbO <sub>2</sub> )	1
Céruse (PbCO <sub>3</sub> , Pb(OH) <sub>2</sub> )	1
Alun	1
Quicklime	1
Verdigris	5
Mercury	1

**Table 4. Zone of inhibition and MIC calculated for the different preparations tested in 96-well plates.** The number of the preparation refers to that of Figure 3. The zone of inhibition is given in mm, as the diameter of the halo around the offset of the remedy (Figure 4), with a standard deviation <10%. The value for tobramycin corresponds to that of 1 µg loaded on the plate. The MIC value represent the lowest concentration of the preparation (diluted in the growth medium) that completely inhibited bacterial growth, and is given in µL/mL. The concentrations tested ranged from 125 µL/mL to 0.125 µg/ml. The MIC for the tobramycin is given as a positive control (in µg/mL)

	<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	<i>Inhib. zone</i>	<i>CMI</i>	<i>Inhib. zone</i>	<i>CMI</i>	<i>Inhib. zone</i>	<i>CMI</i>
<b>Control 1</b>	16 mm	15 µg/ml	18 mm	15 µg/ml	18 mm	15 µg/ml
<b>Preparation 3</b>	18 mm	7.5 µg/ml	12 mm	15 µg/ml	20 mm	15 µg/ml
<b>Control 2</b>	-	125 µg/ml	-	125 µg/ml	-	>125 µg/ml
<b>Preparation 4</b>	20 mm	30 µg/ml	13 mm	15 µg/ml	16 mm	15 µg/ml
<b>Preparation 5</b>	16 mm	15 µg/ml	13 mm	15 µg/ml	16 mm	7.5 µg/ml
<i>Tobramycin</i>	<i>12 mm</i>	<i>0.3 µg/mL</i>	<i>12 mm</i>	<i>0.5 µg/mL</i>	<i>28 mm</i>	<i>0.5 µg/mL</i>



Figure 1.



**Figure 2**



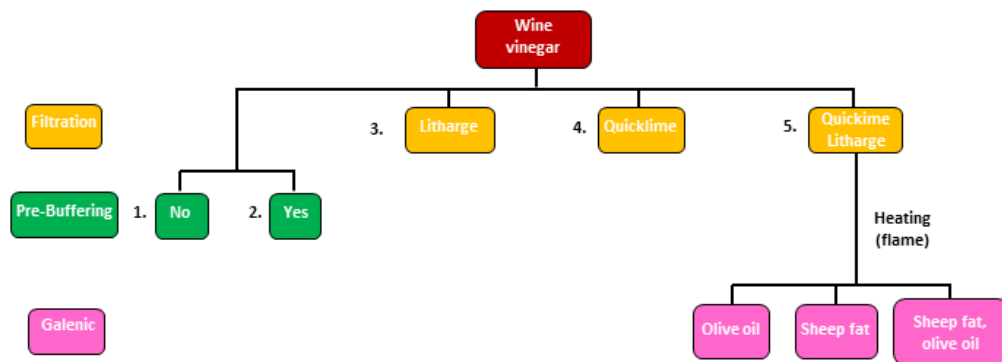


Figure 3

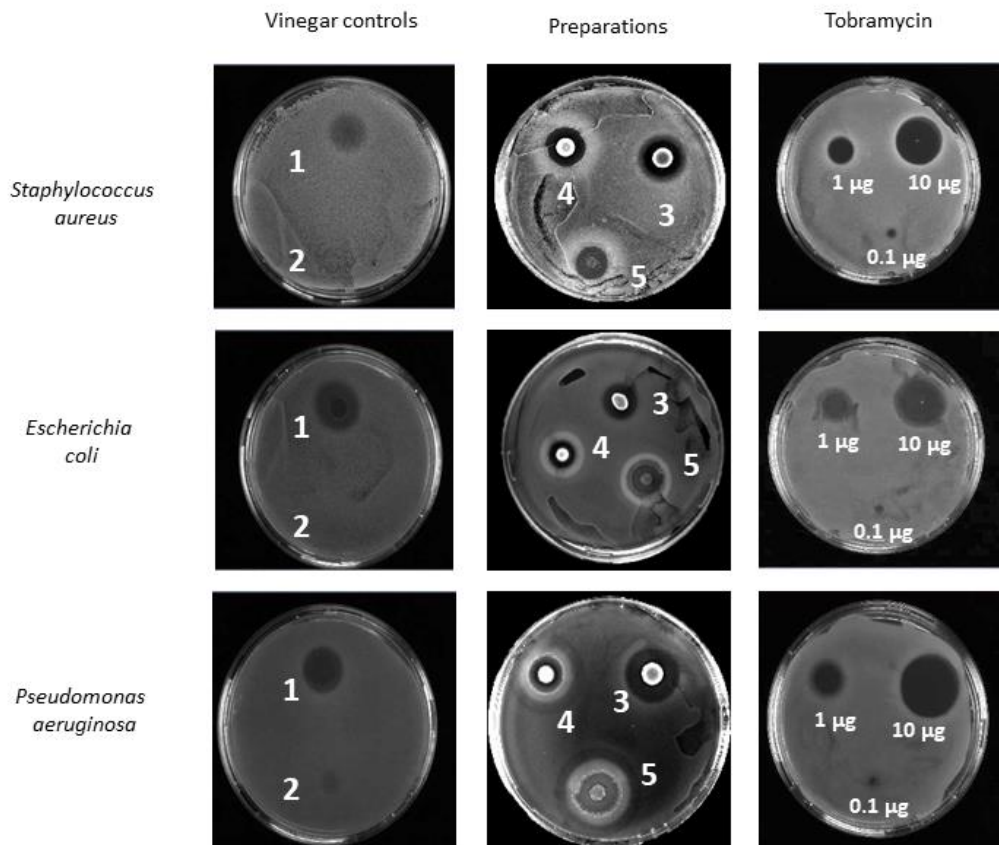


Figure 4

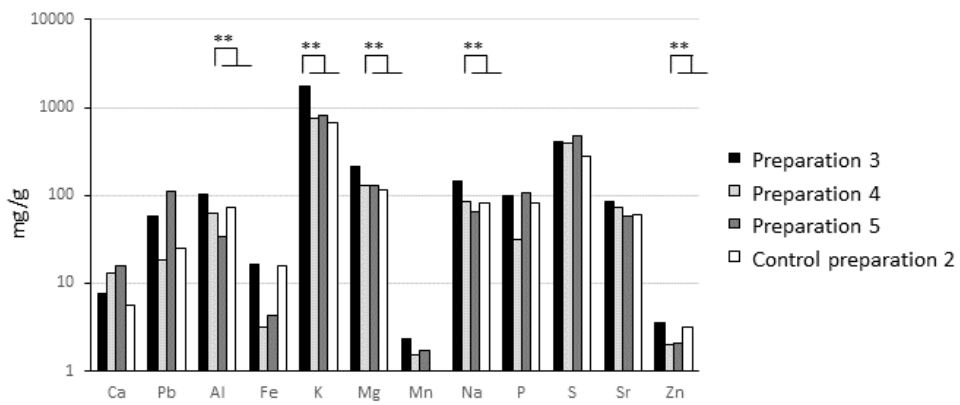


Figure 5

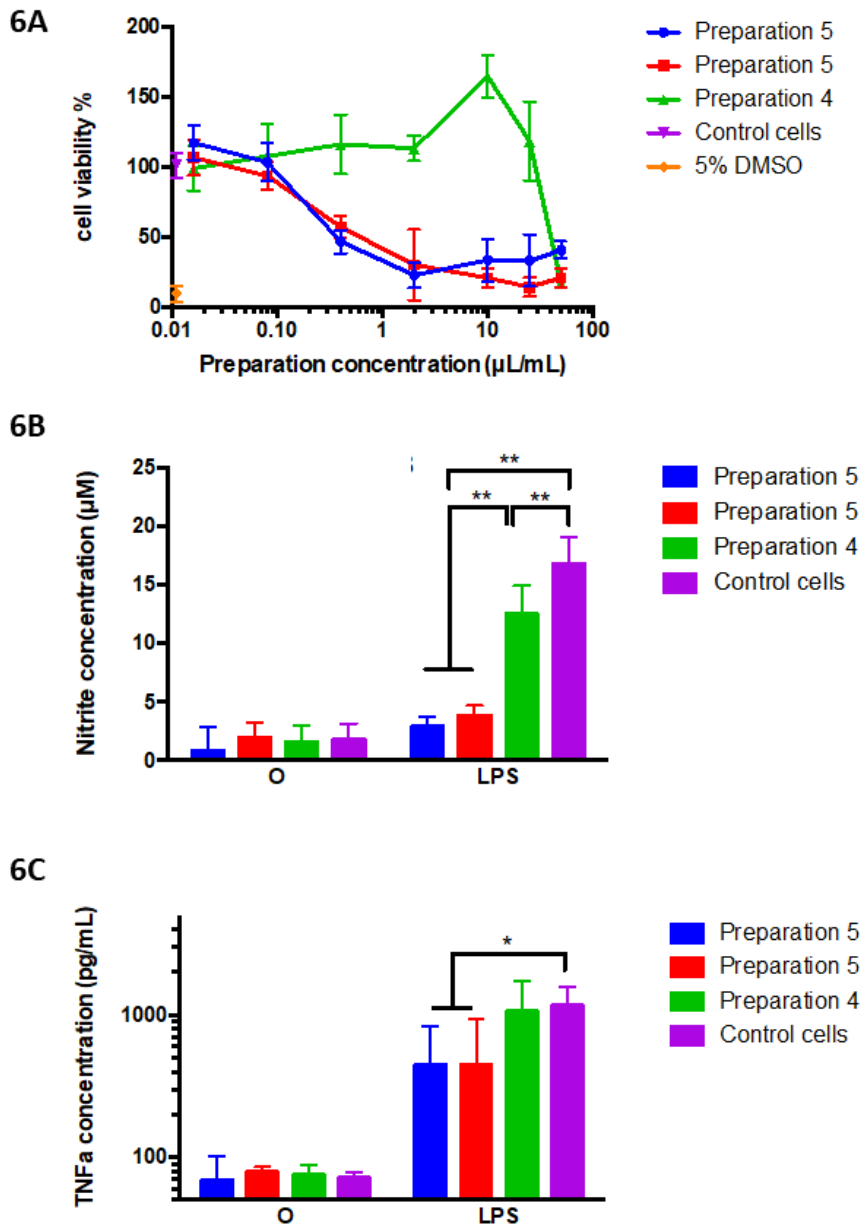


Figure 6