



Empirical Observation of Akhlāt: An Experimental Study

Dr. Mohsin Masood ¹, Dr. Ataullah Fahad ^{1*}, Dr. Mohammed Yasir ²

¹ Assistant Professor, Department of Ilmul Amraz, Faculty of Unani Medicine, AMU, Aligarh

² Assistant Professor, Department of Molajat, Dr MIJ Tibbia Medical College, Mumbai

Article Info:



Article History:

Received 20 April 2025

Reviewed 04 June 2025

Accepted 30 June 2025

Published 15 July 2025

Cite this article as:

Masood M, Fahad A, Yasir M, Empirical Observation of Akhlāt: An Experimental Study, Journal of Drug Delivery and Therapeutics. 2025; 15(7):29-32

[DOI: <http://dx.doi.org/10.22270/jddt.v15i7.7264>](http://dx.doi.org/10.22270/jddt.v15i7.7264)

*For Correspondence:

Dr. Ataullah Fahad, Assistant Professor, Department of Ilmul Amraz, Faculty of Unani Medicine, AMU, Aligarh

Abstract

The Unani System of Medicine is rooted in the humoral theory, which posits that the equilibrium of four Akhlāt (humours) is essential for maintaining health, while their imbalance leads to disease. This study aims to empirically validate the existence of these humours through an experimental approach. Blood samples were subjected to various conditions, including clotting, the addition of hot water, and centrifugation, to observe distinct layers corresponding to the classical descriptions of Akhlāt.

The experiment demonstrated partial confirmation of traditional claims. The upper frothy or yellowish layer was identified as *Şafrā'*, while the lower dark red layer appeared to be a mixture of *Dam* and *Sawdā'*. The addition of hot water revealed an egg-white-like transparent layer, potentially representing *Balgham*. However, differentiation of *Sawdā'* remained challenging, likely due to limitations such as sample size, methodology, and the absence of classical phlebotomy techniques.

This pilot study highlights the need for further research with refined methodologies, larger sample sizes, and quantitative comparisons. Correlating humoral imbalances with haematological and biochemical parameters could lead to the development of objective diagnostic criteria, thereby advancing the scientific foundation of the Unani system of medicine. The study highlights the continued relevance and potential integration of the humoral theory with modern biomedical sciences.

Keywords: Akhlāt, Humour, Dam, *Şafrā'*, *Balgham*, *Sawdā'*

INTRODUCTION

The concept of four Akhlāt (humours) has been a cornerstone of Unani medicine, originating from the humoral theory proposed by Hippocrates and later expanded by Galen. This theory postulates that the human body consists of four fundamental humours: *Dam* (blood), *Şafrā'* (yellow bile), *Balgham* (phlegm), and *Sawdā'* (black bile), with their balance determining an individual's health and temperament¹. The classical Unani scholars have long held that when venous blood is drawn and allowed to settle, it naturally separates into distinct layers, visually representing these four Akhlāt.

To empirically validate this claim, several historical experiments have been conducted. One notable experiment involves drawing blood via venesection (Fasd) and observing its natural stratification over time. Unani physicians have recorded that the uppermost frothy yellow layer corresponds to *Şafrā'*, while the lower clotted portion represents *Dam*. Further, by adding hot water, two additional components emerge: a middle layer resembling egg white, identified as *Balgham*, and a bottom dark sediment considered *Sawdā'*. A modern replication of this experiment was performed in 1921 by Robin Fahraeus, a Swedish physician, who demonstrated

a similar four-layer separation of blood, leading to the establishment of the erythrocyte sedimentation rate (ESR) as a clinical measure².

The relevance of Akhlāt in modern medical science remains a topic of ongoing exploration. Various researchers have attempted to correlate the four humours with contemporary biomolecular components. *Sawdā'*, for instance, has been linked to calcium and phosphorus in bone metabolism, while *Dam* is associated with hemoglobin, the oxygen-carrying component of blood. Proteins, which are an integral part of various physiological functions, are often categorised under *Balgham*. However, the classical colour-based classification of Akhlāt remains a subject of debate, as some hormones and biochemical substances exhibit properties that align more with *Şafrā'* despite their white colouration.

This study aims to experimentally validate the existence of four Akhlāt by observing the separation of blood under controlled conditions. By employing simple laboratory techniques, we seek to replicate the classical claims and assess their alignment with contemporary haematological principles. Through this empirical approach, we hope to bridge traditional Unani medicine

with modern scientific understanding, fostering further research into the physiological significance of the four humours.

Modern explanation of Akhlāt

The Unani System of Medicine bases its approach on the humoral theory, which explains its diverse pathophysiology. According to Hippocrates, the body's health is maintained when the four Akhlāt remain in appropriate balance. Conversely, the onset of diseases occurs when dyscrasia, imbalance, or qualitative variations occur in these humours^{3,4}.

Experimental validation of the existence of four Akhlāt:

Several Unani physicians have reported an experiment based on Hippocrates' theory that when blood is drawn into a vessel through venesection (Fasd) and left undisturbed, it is separated into distinct layers. The upper yellowish or frothy layer was identified as *Şafrā'*, while the remaining clotted lower layer was called Dam. When hot water is added to the vessel, two additional components, a middle layer resembling egg white and a lower blackish sedimented part known as Bhalgam and Sawdā', respectively, are also observed^{5,6,7,8,9}.

Subsequently, this experiment was replicated by Robin Fahraeus in 1921, a Swedish physician who proposed the concept of erythrocyte sedimentation rate (ESR). In his experiment, blood drawn into a transparent glass container and left undisturbed for about an hour displayed four distinct layers: a dark clot at the bottom representing "black bile", a layer of red blood cells above it representing "blood", a whitish layer of white blood cells above representing "phlegm", and finally, a clear yellow serum at the top representing "yellow bile"¹.

Four Akhlāt and Modern Science:

Before the advent of modern medicine, the humoral theory served as the fundamental framework for medical understanding and teachings for over two millennia¹⁰. In the recent past, in an attempt to correlate with modern biomolecular concepts, various scholars associated different Akhlāt with distinct components of human fluids. In one study, Sawdā' is believed to be rich in calcium and phosphorus, serving as a nutrient for bones. Dam is related to haemoglobin at the molecular level. It is described as *Ḩāmil-i-Rūḥ* (carrier of oxygen) in classical literature and is called *Khilṭ Ahmar* (red humour). The bright red color is because of oxygenated blood. Further, haemoglobin has a protein component (attributed to *Khilṭ Balgham*) and an iron ion (attributed to *Khilṭ Sawdā'*)¹¹.

Proteins are categorized within *Khilṭ Balgham*. According to Unani literature, Balgham is formed when consuming *Bārid Raṭb* (cold wet) foods like curds, yoghurt, and buttermilk, which are considered good protein sources. However, it would be questionable to include haemoglobin in *Khilṭ Balgham* solely based on its proteinaceous nature¹¹.

Some researchers believed Hippocrates' categorisation of Akhlāt based on colour is partially acceptable, as all

fluids with similar colour do not exhibit similar Mizāj and functions. For instance, many hormones and body secretions are classified under: Balgham category due to their white colour range. However, these secretions, e.g. catecholamines, thyroxine, acetylcholine, histamine, serotonin, prostaglandin, etc., exhibit stimulant and irritant properties associated with the *Ḩarārat* and *Yubūsat*, resembling the characteristics of *Şafrā'*. These substances could be appropriately categorized under *Şafrā'* (yellow humour) based on their physiological properties¹².

The human body has a special type of fluid which forms the black substance in the human body, such as melanin and black iron components like ferritin, hemosiderin, porphyrin, etc¹³.

As highlighted by medical historians, the humoral theory is considered a precursor to the understanding of metabolic disorders initially described by Garrod. Its significance in medical science is underscored by the connection to the blood group system pioneered by Landsteiner, seen as an extension of the humoral theory. The theory's ongoing relevance is evident in the discovery of messenger substances like hormones, growth factors, and neurotransmitters. References to individuals as having good or bad humour and descriptions of temperaments such as sanguine, phlegmatic, bilious, or melancholic reflect the enduring impact of the humoral theory on societal language. Even in contemporary medical terminology, remnants of the theory persist, using humoral immunity to describe substances like hormones and antibodies circulating in the bloodstream. Additionally, blood dyscrasia is still employed to denote a blood disease or abnormality¹⁰.

In summary, the Humoral Theory, originating with ancient Greek philosophers like Hippocrates and Galen, has an elegant and logical explanation of the human physiological constitution and pathological manifestations.

MATERIALS AND METHODS

- Place of Study: The study was conducted in the Department of Ilmul Amraz, Ajmal Khan Tibbiya College, AMU, Aligarh, in 2023.
- Study Subject: One healthy volunteer was included in the study after informed consent
- Study Design: Observational study of the physical appearance of four Akhlāt
- Instruments Used: Petri dish (90mm), A syringe with needle, Glass test tube (10ml), Spirit lamp, Cotton swab, Tourniquet, Stopwatch

Procedure:

15 ml of venous blood was drawn from the basilic vein through venipuncture with a sterile syringe. The blood was drawn in different tubes and dish as follows;

- 3 ml of blood was kept in two different test tubes, A & B
- 3 ml of blood was drawn in test tube C, already containing 10 ml of warm water

- 3 ml of blood was drawn in test tube D
- 3 ml of blood was kept in a petri dish

All these samples were left untreated in a stable place at room temperature for 5 minutes. After this, 3 mL of hot water was added to test tube B and left undisturbed for an additional 5 minutes. Test tube D is centrifuged at 3000 rpm for a few minutes. All the test tubes were inspected for any morphological change, and observations were noted.

OBSERVATION AND RESULTS

The following are the observations noted during the examination according to the methods described;

- Initially, the blood drawn was uniform red and normal in consistency.
- *On simple clotting:* In test tube A, two distinct layers were observed after 10 minutes: a yellowish, frothy layer above and a dark red-coloured layer below.

- *On addition of hot water to clotted blood:* In test tube B, after 10 minutes (blood clotted in 5 minutes and again kept 5 minutes after adding 3 ml hot water to the clotted blood), a transparent layer having an egg white-like appearance was observed above, yellowish layer in the middle and a dark red clotted sediment was observed at the bottom.
- *On adding fresh blood to hot water:* In test tube C (where blood was directly added to hot water), after 10 minutes, most of the blood had sedimented as a dark red layer, except for a thin layer at the top with a lighter red colour.
- *On centrifugation of fresh blood:* In test tube D, after 10 minutes of centrifuge, a dark blackish-red colour sedimented, and a yellowish layer appeared above.
- *On simple clotting in a petri dish:* In a petri dish, after 10 minutes, two layers were observed: a dark red sediment and a light red, watery layer above it.



Figure 1.1 Test tube A
(On simple clotting)



Figure 1.2. Test tube B
(On addition of hot water in
clotted blood)



Figure 1.3 Test tube C
(On adding fresh blood in hot
water)



Figure 1.4 Test tube D
(On centrifugation of fresh blood)



Figure 1.5 Petri Dish
(On simple clotting)

The interpretation of these observations regarding Akhlāt is mentioned in the discussion section.

While investigating Akhlāt, we designed the experiment in various ways to observe these four aspects of Akhlāt. However, it remained a little tricky for us to arrive at the similar findings mentioned by Allama Gheelani and other sages. On simple clotting and on addition of hot water in clotted blood, we observed two layers. The upper frothy or yellowish layer may be Ṣafrā and the lower dark red coloured layer a mixture of Dam and Sawdā (Figure 1.1 & 1.2). The egg white-like transparent layer observed only after the addition of hot water might be Balgham (Figure 1.2). On centrifugation of fresh blood, yellowish and dark red layers were found but not a distinct white layer, which is usually observed during packed cell volume examination, possibly because the sample was not anticoagulated blood. On adding fresh blood in hot water, only a thin layer of light red colour is separated without any other yellowish or frothy layer. A similar case involved simple clotting in a petri dish. This experiment partially confirms the classical claim. The reason behind this may be the small quantity of blood, the usage of normal-sized needles for drawing blood instead of the classical method of Fasd (phlebotomy) and the sampling of only one subject. This pilot work might stimulate researchers to investigate further the traditional claim eliminating all these possible reasons with better experimental design. In the future, studies with quantitative comparisons will help in understanding the proportion of Akhlāt in the body in both physiological and pathological conditions.

DISCUSSION

An experiment is designed in different ways to observe these four Akhlāt clearly. However, it remained a little tricky for us to arrive at the similar findings mentioned by Allama Gheelani and other sages. We observed Dam, Ṣafrā, and Balgham but could not differentiate Sawdā from Dam. The reason behind this may be the small quantity of blood, the usage of a normal-sized needle for drawing blood instead of the classical method of Fasd (phlebotomy) and the sampling of only one subject. This pilot work may stimulate researchers to investigate the traditional claim further, eliminating these possible reasons with a more rigorous experimental design. In the future, studies with quantitative comparisons will help in understanding the proportion of Akhlāt in the body in both physiological and pathological conditions.

CONCLUSION

The present study aimed to experimentally validate the classical Unani concept of four Akhlāt (humours) through simple laboratory techniques. The observations partially aligned with traditional descriptions, with identifiable layers suggestive of Dam, Ṣafrā, and possibly Balgham under certain conditions. However, a distinct separation

of all four humours, particularly Sawdā', was not consistently observed. These limitations may be attributed to methodological constraints, such as the use of a small blood volume, the application of modern venipuncture techniques in place of classical Fasd, and a single-subject sample. Despite these challenges, the experiment offers preliminary empirical support for the humoral theory and highlights the need for further research. Future studies with refined methodologies, larger sample sizes, and quantitative biochemical analyses could provide deeper insight into the physiological relevance of the four Akhlāt and help bridge classical Unani wisdom with modern scientific paradigms.

Informed Consent: Written informed consent was obtained from the volunteer.

Financial support and sponsorship: Nil

Conflict of Interest: There is no conflict of interest

REFERENCES

1. Sina, I (Avicenna). Al-Qanun fi al-Tibb (The Canon of Medicine) (Trans. L. Bakhtiar). Kazi Publications. 1999
2. Hart GD. "Descriptions of blood and blood disorders before the advent of laboratory studies" (PDF). Br. J. Haematol. 2001;115(4):719-28. <https://doi.org/10.1046/j.1365-2141.2001.03130.x> PMid:11843802
3. Lone, A. H., Ahmad, T., Anwar, M., Sofi, G. H., Imam, H., & Habib, S. Perception of health promotion in Unani herbal medicine. *Journal of Herbal Medicine*, 2012;2(1):1-5. <https://doi.org/10.1016/j.hermed.2012.02.003>
4. Israili A. H. Humoral theory of Unani Tibb. *Indian journal of history of science*, 1981;16(1):95-99.
5. Kabeeruddin, H. M. (1954). *Kuliyat Nafisi Tarjuma wa Sharha* (pp. 59). Idara Kitab Us Shifa.
6. Kabeeruddin, H. M. (1916). *Ifaaede Kabeer* (pp.48-49). Quomi Council Barae Farogh urdu zabaan, New Delhi
7. Kabeeruddin, H. M. (1946). *Kitabul Akhlat. Daftar-ul-Maseeh Qaraul Bagh*, Delhi.
8. Chandpuri, K. (1984). *Moajuzul Qanoon*. Quomi Council Barae Farogh Urdu Zubaan, New Delhi
9. Qarshi, A. A. (2018). *Afad-e-Kabeer* Translated by Mohd. Kabeeruddin. Idara Kitab us Shifa, New Delhi.
10. Bhikha, R., & Glynn, J. The theory of humours revisited. *Int J Dev Res*, 2017;7(09):15029-34.
11. Rizvi, A., & Sherani, F. S. Physiological Roles, not Structure will Categorize Placement of Molecules in Humours (Akhlāt) in Unani Medicine: The Case of Human Haemoglobin. *Advanced Chemicobiology Research*, 2022;72-76. <https://doi.org/10.37256/acbr.1220221465>
12. Jahan M, Riaz A, Nuzhat S. Understanding the Physiological basis of Khilt-e-Safra (Yellow bile) in Unani system of medicine; An Overview. 2022.
13. Ghazali, S. A. Review on Khilt e Sauda Khilt e Aswad (Black Bile or Melanchole). *European Journal of Biomedical and Pharmaceutical Sciences*, 2019;8(5):212-214.