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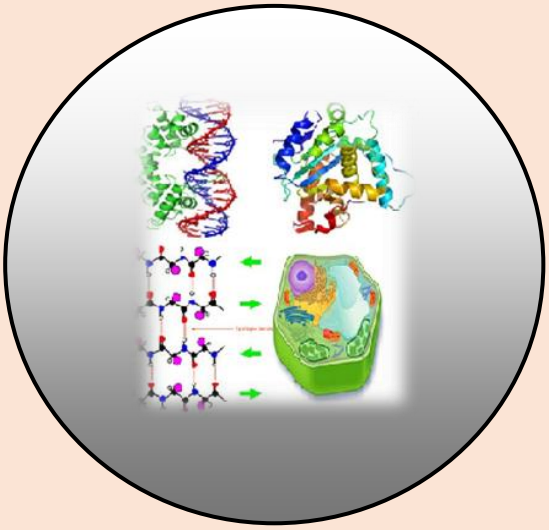

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RESEARCH PAPER

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Diagnosis of Different Diseases by Spectrophotometry of Urine – A Hypothesis

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ABSTRACT

Introduction
Imtehan-e-boal (urine examination) is one of the most important methods of Unani System of medicine which has been used as a diagnostic tool since ages. Imtehan-e-boal includes imtehan-e-loan-e-boal that is discussed under physical examination of urine. This work presents a hypothesis of using spectrophotometric techniques for determining exact optical transmittance of urine and thus exact urine colour. This will help in diagnosis of various diseases.
Type of study: descriptive research
Procedure: this hypothesis suggests the following steps of diagnosis of diseases by spectrophotometry of urine:

1. *Various reference books of qadeemilmulamraz (ancient pathology) shall be reviewed for understanding the concept of imtehan-e-loan-e-boal in detail.*
2. *Various normal and abnormal types of urine shall be noted as classified on the basis of urine colour.*
3. *Various pathological and physiological conditions indicated by different colours of urine shall be noted as mentioned in classical text.*
4. *The optical density of different urine samples is determined. The reciprocal of optical density can be obtained. This is the transmittance of the urine. The wavelength transmitted by the sample would be the colour of urine.*
5. *After determination of exact colour particular body state (health or disease) can be predicted.*

Expected outcome: after repeating this procedure on a large sample size the wavelength of urine transmitted during spectrophotometry in a particular disease can be fixed. This will be a strong tool in diagnosis of that disease.
Keywords: Imtehan-e-boal, Imtehan-e-loan-e-boal, Spectrophotometry, Transmittance and Absorbance.

INTRODUCTION

Unani scholars use three tools for the diagnosis of various disease:

1. Pulse examination (*imtehan-e-nabz*)
2. Urine inspection (*tafsira-e-boal*)
3. Stool inspection (*imtehan-e-baraz*)

The art of urine inspection, or uroscopy (*tafsira-e-boal*), is an important part of traditional Greco-Arabic, or Unani Medicine. Avicenna devotes considerable space to it in his Canon of Medicine. Case studies in the Hippocratic writings show that careful note was made of the patient's urine. The medical treatises of Unani physicians on *tafsira-e-boal* like Ibn Sina, or Avicenna became the basis for medical practice in Europe in the high middle Ages.

As long as there is life, there is *pepsis,* or digestion and metabolism. Elimination, or the excretion of unusable wastes, is the final stage of *pepsis;* all metabolic processes generate waste. The wastes generated by the metabolic processes of the body can tell the physician a lot about these processes. And so it is with urine, which is the principal liquid waste of the body; the urine provides the physician with a window into the inner metabolic processes of the organism. The wastes of the First Digestion are excreted via the stool. But the wastes from the Second through Fourth stages of digestion, from the initial coction and generation of the humors in the liver until their final assimilation and transmutation into living tissue, are excreted mainly via the urine. Besides excreting liquid wastes, the kidneys, through the production and discharge of urine, also work to balance the humors and chemistry of the bloodstream. Any excesses or superfluities are thrown off, while that which is in short supply is conserved. But what is most apparent and visible in the urine are those superfluities which are being eliminated.

Although careful inspection of the urine will yield much information about many metabolic processes of the body, the urine most directly indicates the state of the liver and the condition of the genitourinary organs and their passages and vessels. Less directly, the urine yields information about the condition of the other organs.

Owing to the importance of urine inspection in unani therapeutics this hypothesis was formulated in the department of Tashreeh Wa Munafeul Aza for make the best implication of our ancient knowledge for the diagnosis of various diseases.

Need of this study: urine examination besides all the precautions is subjected to individual observations leading to high probability of manual errors and lack in precision. Therefore, there was a need to employ modern technological methods to minimise manual errors and individual variations. Hence, this hypothesis of using spectrophotometry for *tafsira-e-boal* was formulated.

Hypothesis:

This hypothsesis suggests that if *tafsira-e-boal* instead of doing naked eye examination, spectrophotometry of urine will be done then the urine colour can be determined with precision. Based upon that various diseases can be diagnosed. This hypothesis suggests the following steps for using spectrophotometry for *tafsira-e-boal:*

1. Various reference books of qadeemilmulamraz (ancient pathology) should be reviewed for understanding the concept of *imtehan-e-loan-e-boal*in detail.

Significance of Inspection of Urine, (tafsira):

Avicenna says in Al Qanoon, "The name "tafsira" is given to "inspection" because it "explains" (tufassir) and makes manifest to the physician; it is an indication or guide (dalil) to the patient's condition".

According to Avicenna, precautions necessary in Collecting the Urine, before forming an Opinion as to its Character are as follows:

1. It must be collected in the early morning. It must not have been kept over from the night before.
2. The person must not have taken either food or drink before passing it.
3. The previous food must have been free from colouring agents like crocus and cassia fistula (these render the urine lemon yellow or. ruddy), and from potherbs (which make the urine a greenish tint), and from salted fish (which renders the urine dark), and from intoxicating wines (which tend to render the colour of the urine similar to themselves).
4. The patient should not have been given an agent which expels some humour (a cholagogue or phlegmagogue) by the urine.
5. The patient should not have undertaken severe exercise or toil, or be in a praeternatural mental state; for in each case the colour of the urine may alter. E.g., fasting, wakefulness, f toil, anger, dread—for all these cause the urine to become more lemon-yellow or redder in tint.

Coitus also alters the urine, rendering it oily. Vomiting and defecation alter both colour and texture of the urine. The same happens if the urine is kept standing a while. "This is why some advise urine not to be left standing more than six hours before examination, for otherwise the significance is altered; the colour changes; the sediment goes partly into solution; and the density increases. Personally Ibn Sina believed that such changes begin within an hour.
6. The whole of the urine should be collected into one single vessel lest anything should be spilt out of it; one should allow it to settle before scrutinizing it.
7. The urinal must be clean. For instance, alkanna will impart its colour to the urine; this is a dye used by some people for tinting their skin and finger-nails.
8. The vessel used for the specimen must be clean, and the previous sample must have been rinsed out of it.
9. The material of which the vessel is made should be clear white glass or crystal.
10. The urine must not be exposed to the sun or wind or freezing cold, until the sediment has separated out and the various characters have properly developed. The settling is not immediate even if the digestive processes are normal.
The sample must be inspected in a light place where the rays do not fall directly upon it, as otherwise the brilliant light would interfere with the colours and give rise to erroneous deductions. The nearer one holds the sample to the eye, the denser does it appears. The further away it is the clearer does it seems. In this way one can distinguish urine from other fluids brought to the doctor in a falsified state.
There is little advantage to be derived from the study of the urine in childhood, and still less in infancy, because their nourishment consists solely of milk, and the very little colouring matter there is in the urine is lost to view; their "nature" is also very feeble in view of the fact that they pass so much time in sleep, which abolishes the evidences of digestion. 605. The first and foremost object of observing the urine is to form an opinion about the state of the liver, the urinary passages and the blood-vessels. The various disorders of these organs are revealed by it. But the most precise information to be obtained is that concerning the functional capacity of theliver.

Basic Parameters of Urine Diagnosis
The physician inspects the urine for the following basic parameter

1) Colour
2) Consistency - degree of thickness or thinness; viscosity
3) Clarity - degree of clearness or turbidity (cloudiness)
4) Sediment - presence or absence, and type of sediment
5) Foam - characteristics of foam formed either initially, or when shaken vigorously
6) Odor - either absence of odor or the presence of strong, offensive or unusual odors

2. Various normal and abnormal types of urine seen in various physiological and pathological conditions of the body ought to be noted:
Generally, tints or shades of urine other than yellow denote more abnormal, morbid conditions. Often, the tint of the urine will indicate the nature of the morbid humor or superfluity.
Basically, the level of color present in the urine indicates the level of Metabolic Heat that the kidneys have at their disposal to ripen and eliminate uric acid and other wastes and superfluous humors from the body.
White or colorless urine generally indicates a Cold temperament prevailing in the organism; there is insufficient metabolic heat to adequately ripen the toxins and other wastes for elimination. Besides coldness, colorless, watery urine can also be due to excessive fluid consumption, and consequently fluid elimination, which is diluting the urine; if such is the case, the volume of urine being passed will usually be copious.
The basic normal color of urine, if it is to have color, is various shades of yellow. This typical yellow color we now know to be due primarily to the presence of uric acid, but it was traditionally considered to be a manifestation of the action of the Metabolic Heat; the greater the heat, the darker the shade of yellow. Galen considered the yellowness of the urine to be a by product or waste product, of the hot, bilious humor in the liver.

Avicenna, in his Canon of Medicine, describes the various possible shades of yellow as: straw yellow, citron yellow, reddish yellow, orange-yellow, flame yellow; saffron yellow, also called perfect yellow; and saffron colored, or bright red.

Straw yellow and citron yellow, Avicenna states, denote a balanced, normal heat level and an equable temperament. Lighter shades of yellow would be colder in temperament.

The darker saffron and flame shades of yellow denote a hotter, bilious temperament. This is particularly true of acute hot natured diseases. With extreme heat, the urine can even be brownish in color. Insufficient fluids can also concentrate the urine and turn it a darker color. And then, a bright yellow colored urine can also be due to the consumption of vitamin supplements.

Obviously, urine tinted red usually indicates the presence of blood, or hematuria; most commonly, there is bleeding in the bladder, kidneys or urinary tract. Some foods, like saffron or beets, may color the urine red.

Brown urine is usually a very concentrated form of yellow, denoting an extremely hot or bilious temperament. Brown urine is usually associated with biliousness, jaundice, or the abnormal charring of bile. The heat level is even greater if the urine burns when being excreted. Urine which is the color of fresh meat washings is a grave sign. It can indicate an excess or superfluity of blood, but most often, it indicates a weakness or unbalanced, bad temperament of the liver; in such states, there is also a weakness of *pepsis,* or digestion, and a dispersion of vitality. Green urine generally indicates a lack of Innate Heat, and an insufficient combustion or coction of the humors. A bright rainbow green is usually indicative of poisoning, and is particularly serious if no sediment is present. A green resembling bronze rust or patina indicates a total extinguishing of the Innate Heat, and is usually terminal.

Black, dark brown, gray or ashen shades of urine are generally associated with black bile. A greenish black denotes the presence of normal black bile. A brownish black is associated with Sanguineous forms of black bile. Black urine that is very dark is a grave sign; it can either indicate a high level of charring, or oxidation; great interior cold; impending death; or a great catharsis of superfluous black bile.

The passing of dark or abnormal shades of urine can be a good sign if it occurs in acute diseases, especially at the time of the crisis, which is a catharsis, or cleansing of morbid matter from the body. If seen at the commencement or at the end of an acute illness, however, it is generally an ominous sign. Dark urine passed in the elderly, or in the chronically ill, does not bode well, and often indicates a great destruction of the internal organs and tissues.

3. Various pathological and physiological conditions indicated by different colours of urine shall be noted as mentioned in classical text:

The Significance of the Colour of the Urine:

I. The degrees of yellowness. Among the shades of yellow colourare: (1) straw-yellow; (2) lemon-yellow; 3) orange-yellow; (4) flame-yellow, or saffron-yellow; that is, a very deep yellow; (5) clear reddish-yellow. All except the first two denote a hot intemperament, in degrees varying with the amount of exercise, pain, fasting, and abstinence from water. The fourth variety denotes predominance of the bilious humour,

II. The degrees of redness, (i) rose-red or roseate; (2) very dark red; (3) purple red, which has a brilliance about it like a certain rose; (4) smoky red or dull red. All these denote dominance of the sanguineous humour, for dullness, of colour points that way. A flame-yellow shows the presence of more "heat" than dull red because it shows there is bilious humour in it, and this is hotter than sanguineous humour. The urine tends to saffron-yellow and flame-yellow in acute maladies described as "burnin "; but if the urine is at all inclined to be clear, it shows a certain degree of '' digestion,' namely that this process has actually begun, but its products have not yet appeared in the substance of the urine. Deepening of colour from lemon-yellowness towards a flame-yellow shade shows that the innate heat is steadily increasing. The colour then ceases to be yellow, and attains a pure clear red. If the urine now begins to clarify it shows that the (pathological) heat is beginning to subside. In acute diseases of a haemorrhagic character, the urine may be tinged with blood without any evident rupture of bloodvessels having occurred.

This would indicate an excessive plethora. A gradual loss of blood by the urine, associated with a bad odour, is a sign to be dreaded because it informs us that there is haemorrhage proceeding from congested parts. I he prognosis is still worse if the urine becomes thinner and more offensive in odour. Admixture of the urine with blood may be a good sign—namely in acute composite fevers—for it show's that crisis is about to take place, and recovery will follow. The only exception is if the urine becomes suddenly transparent (its colour becoming normal, i.e.) before the crisis is due. Such a phenomenon would be a forerunner of a relapse. But thin urine appearing before the crisis may be equally unfavourable unless the change has been gradual and progressive. In jaundice, if the urine becomes of a deeper red until it is nearly black, and its stain on linen can no longer be removed, it is a good sign;—the better the deeper the red. But if the urine becomes white or slightly reddish, and the jaundice is not subsiding, the advent of dropsy is to be feared. Fasting is among the conditions which render the urine, high-coloured and of marked acridity.

IV The degrees of green colour: (i) A Colour approaching that of pistachios; (2) the colour of verdigris; (3) rainbow green (4) emerald green; (p) leek-green. The first denotes a cold intemperament, as do all things the shade of whose green is not (2) or (5). These (2, c) denote extreme combustion, but (5) is not as unhealthy as (2). If it should be met with after-' physical labour it denotes "spasm." A green coloured urine in adolescence points to the same condition. Rainbow green usually denotes an extremely cold intemperament. In this respect it comes next to (1). Some say that it shows that poison was present in the fluid taken as drink, and that if there be sediment present there is a hope of recovery; if no sediment, death is likely to take place. Verdigris green colour of urine forewarns of death (destruction of innate heat).

V. The degrees of blackness: (i) Dark urine approaching blackness, through a saffron colour. This occurs in jaundice, for instance. It denotes (a) denseness and oxidation of the bilious humour; (b) atrabilious humour derived from bilious humour; (c) jaundice. (2) Deep-brown-black. This shows the presence of sanguineous atrabilious humour. (3) Greenish-black. 4 his shows the dominance of pure atrabilious humour. (Speaking generally) dark or black urine denotes (a) extreme oxidation ; (b) great cold; (c) extinction of the innate heat (i.e. death) ; (d) crisis ; (e) evacuation whereby the effete substances from the atrabilious humour are expelled. The details about each of these are:

(a) dark urine due to extreme oxidation is recognized by its causing scalding, and being previously yellow or red. The sediment is discrete (not coherent), not homogeneous, discontinuous, not very dark, but tending to a saffron, lemon-yellow, or dark brown. If the colour of the sediment tends to be lemon-yellow, it strongly suggests jaundice.

(b) When darkness of the urine is due to great cold, the urine would previously be tending to a green tint or a livid tint. The sediment is here slightly coherent, and looks dry, and is more purely black in colour. If dark urine is also very offensive, it shows that the temperament is hot. If it be odourless, or has only a slight odour, it shows that the temperament is cold. This is because no odour emanates from urine unless the innate heat overrules the cold.

(c) When darkness of urine is due to extinction of the innate heat this is recognized by the dispersion of vitality.

(d) When the darkness arises from a critical change in a fever one of the following conditions may be supposed: the termination of a quartan fever; the resolution of a splenic disease; the termination of a fever associated with the atrabilious humour; the termination of a fever prevailing by night and by day ; subsidence of pains in the back and womb ; retained menses ; retained blood in a case of piles both the latter two occurring especially when nature is assisted by it. It occurs in women in whom the menses are retained, because the effete matters of the blood cannot be disposed of by nature.

4. Determine exact colour by transmittance of urine samples using spectrophotometre

Spectrophotometre is an apparatus for measuring intensity of light in a part of the spectrum, as transmitted or emitted by particular substances.

Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength. Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits. Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications, industrial applications, etc).

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. The visible light region consists of a spectrum of wavelengths that range from 700 nanometers (nm) to 400 nm. All the wavelengths of different colours lie within this range.

In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed color. For instance, a solution sample that absorbs light over all visible ranges (i.e., transmits none of visible wavelengths) appears black in theory. On the other hand, if all visible wavelengths are transmitted (i.e., absorbs nothing), the solution sample appears white. If a solution sample absorbs red light (~700 nm), it appears green because green is the complementary color of red. Visible spectrophotometers, in practice, use a prism to narrow down a certain range of wavelength (to filter out other wavelengths) so that the particular beam of light is passed through a solution sample.

Determine the transmittance of urine sample for the whole range of visible spectrum i,e. 700-400nm as per following steps:

1) Turn on the spectrophotometer. Most spectrophotometers need to warm up before they can give an accurate reading. Turn on the machine and let it sit for at least 15 minutes before running any samples.
2) Clean the cuvettes or test tubes. Rinse each cuvette thoroughly with deionized water.Take care with cuvettes as they can be quite expensive. When handling the cuvette, avoid touching the sides the light will pass through it.
3) Load the proper volume of the sample into the cuvette. Some cuvettes have a maximum volume of 1 milliliter (mL) while test tubes may have a maximum volume of 5 mL. As long as the laser producing the light is passing through the liquid and not an empty part of the container, an accurate reading is ensured.New tip should be used for each sample to prevent cross-contamination.
4) Prepare a control solution. Known as a blank, the control solution has only the chemical solvent in which the solute to be analyzed is dissolved in. The blank is the same volume as the solution to be analyzed and kept in the same kind of container.
5) Wipe the outside of the cuvette. Before placing the cuvette into the spectrophotometer to avoid interference from dirt or dust particles. Using lint free cloth, remove any water droplets or dust that may be on the outside of the cuvette.
6) The visible light region consists of a spectrum of wavelengths that range from 700 nanometers (nm) to 400 nm. Therefore, choose and set the wavelength of light within this range to analyze the sample with. Use a single wavelength of light (monochromatic color) to make the testing more effective. The color of the light chosen should be one known to be absorbed by one of the chemicals thought to be in the test solute. Set the desired wavelength according to the specifications of spectrophotometer.
7) Calibrate the machine with the blank. Place the blank into the cuvette holder and shut the lid. On an analogue spectrophotometer, there will be a screen with a needle that moves based on the intensity of light detection. When the blank is in the needle move to the right. With the blank still in the machine, move the needle to zero using the adjustment knob.Digital spectrophotometers can be calibrated in the same way, they will just have a digital readout. Set the blank to 0 using the adjustment knobs.When you remove the blank, the calibration will still be in place. When measuring the rest of your samples, the absorbance from the blank will automatically be subtracted out.Be sure to use a single blank per session so that each sample is calibrated to the same blank.
8) Remove the blank and test the calibration. With the blank removed the needle should stay at 0 (zero) or the digital readout should continue to read 0. Place the blank back into the machine and ensure the needle or readout doesn't change. If the machine is properly calibrated with blank, everything should stay at 0.If the needle or readout is not 0, repeat the calibration steps with the blank. If you continue to have problems, seek assistance or have the machine looked at for maintenance.

9) Measure the absorbance of experimental urine sample. Remove the blank and place the experimental sample into the machine. Slide the cuvette into the designated groove and ensure it stands upright. Wait about 10 seconds until the needle is steady or until the digital numbers stop changing. Record the values of % transmittance and/or absorbance.The absorbance is also known as the optical density (OD).
10) Repeat the reading for each individual sample at least 3 times and average them together. This ensures a more accurate readout.
11) Repeat the test with successive wavelengths of light. Urine sample may have multiple unknown compounds that will vary in their absorbance depending on wavelength. To eliminate uncertainty, repeat the readings at 25 nm intervals across the spectrum. This will allow you to detect other chemicals suspected to be in the solute.
12) Calculate the transmittance and absorbance of the sample. Transmittance is how much of the light that passed through the sample reached the spectrophotometer. Absorbance is how much of the light has been absorbed by one of the chemicals in the solute
13) The transmittance (T) is found by dividing the intensity of the light that passed through the sample solution with the amount that passed through the blank. It is normally expressed as a decimal or percentage. $T = I/I_0$ where I is the intensity of the sample and I_0 is the intensity of the blank.The absorbance (A) is expressed as the negative of the base-10 logarithm (exponent) of the transmittance value: $A = -\log_{10}T$.[6] For a T value of 0.1, the value of A is 1 (0.1 is 10 to the -1 power), meaning 10% of the light is transmitted and 90% is absorbed.
14) Plot the absorbance values versus the wavelengths on a graph. The absorbance value is plotted on the vertical y-axis against the wavelength of light used for a given test plotted on the horizontal x-axis. Plotting the maximum absorbance values for each wavelength of light tested, produces the sample's absorbance spectrum and identifies the compounds making up the test substance and their proportions.The wavelength at which the transmittance will be maximum should be noted. This wavelength will indicate the exact colour of urine. For example if the wavelength at which urine showed maximum transmittance is 570-590nm; then the urine sample is of yellow variety and so on.

5. After determination of exact colour particular body state (health or disease) can be diagnosed:
For example if the urine sample is of yellow variety it indicates a hot intemperament, exercise, pain, fasting, abstinence from water and predominance of the bilious humour.

DISCUSSION AND CONCLUSION
After repeating this procedure on a large sample size the wavelength of urine transmitted during spectrophotometry in a particular disease can be standardized. This will be a strong tool in diagnosis of that disease.

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