Impact of Natural Plant on characterization of Nano Biomaterial for Blood Toxicology

Ekhlas Majeed Hameed, Asmaa Hadi Mohammed, Baida Mohsen Ahmed
Department of Physics, College of Science, Mustansiriyah University, Baghdad, Iraq,
alqaem1440@gmail.com

Abstract:
In this study, the effect of adding natural plants on the properties of nanomaterial’s to treat blood poisoning caused by exposure to continuous doses of nuclear radiation has been studied. Nanomaterials were prepared in simple, inexpensive physical and chemical methods. The treatment results showed a high efficacy with a short period of time

Keywords: Natural plant, Blood toxicology, Nano biomaterial.

Introduction

Herbs already used throughout the ages of humaneness as the base of classical therapy and even serve as basic of advances of medical field. And over 3/4 of populations in the countries which regards resources-confined, they dependent on medicinal herbs for the medicinal benefits, that what said (WHO) in one report, as around 60 percent of communities are unable to reach and/or paying for modern medicines [1]. In line with the latest advances in the field of healthy live, in the employ of herbs, vegetables and fruits as a source of food and medical supplies is now resurgent [2]. Nowadays, the medicinal plant has been significantly raised of usage for many illnesses, not just because of the simple getting and cheap price, but as well because of the thought that homeopathy products have lesser adverse impact comparing with artificial drugs [3]. (Ranunculaceae plant) and black seed were already remains two of the largest most cherished nutritionally dense herbs in world memory. The black seed or (NS) seed was used for centuries in various cultures on the earth to cure different animal and humaneness illnesses. To date, several studies have been shown that the black seed (NS) and its major component ingredient (thymoquinone) are very beneficial medicinally toward different diseases, as well as for various chronic diseases: psychological and cognitive diseases, heart disease, tumors, some liver diseases, immune disorders and birth defects, also different infectious illnesses caused by bacteria, microbial, viral infections. Given the few studies carried out up to now but black seed is hopefully effectiveness versus HIV / AIDS can be investigated as an additional treatment choice for this pandemic epidemic, after demonstrating its maximum therapeutic activity. In addition, this remunerated seed's strong anti-oxidant item has lately yielded raising awareness regarding its possible impact as a food additive with fewer side effects. Moreover, it enhances their impact when paired with specific artificial chemotherapy drugs, which leads in a decrease in the amount of effect correspondingly employed medicines with improved effectiveness and least and/or no toxicity. Seeds of NS have been attributed a variety of pharmaceutical and bio pharmacy characteristics [4]. It is a natural herb from families of ranunculus (Ranunculaceae), growth one time yearly and its height around (20-60 cm) or (8-24 inches), distributed in south-western Asia and regions of the Mediterranean and Africa. The spaced buds carry nice leaves, which are heavily divided, and the herbs have an advanced root system. The dull blue or candid flowers are filled with five petals, many stamens and 5 or 6 long and straight merge carpels. The black buds or pyramid - shaped pieces are carried in a five or six segmented capsule, each ending in an extended dropping. The herbs can implant and rapidly growth in a wide range of soils, getting dense herbs in certain areas [5-6]. In addition, thymo-quinone (TQ) is the main bio-active component of the
essential oil of Nigella Sativa (NS) seeds, where (TQ) is the phyto-chemical (C10H12O2) component of the NS seed oil. Work on TQ's anti-cancer activity versus many tumour cell streaks and animal studies have been studied extensively in vitro and in vivo. As ending yield, work has provided a significant amount of knowledge, thus leading to good comprehensive of the anti-tumorigenic impact of this product. So it is reasonable for TQ to switch from bench analysis to medical experiments [7]. The oil of seed has been measured using in vitro tests like DPPH, ABTS, nitric oxide, hydrogen peroxide, and complete anti-oxidant scavenging potential for some of its organic chemicals and anti-oxidant properties. The oil of seed contains a higher portion of fatty acids and displays anti-oxidant action that is useful for therapeutic agent. The table below shows some of the main components of NS, such as eicosyne, linoleic acid, palmitic acid, saturated aliphatic fatty acids, fatty acids and monoterpane hydro-carbons, and includes the constituent’s components of alkanes and sesquiterpene hydrocarbons [8, 9].

**Method Section**

**TiO₂ nanoparticles**

- Chemical method by mixed (1100 ml) of titanium chloride (TiCl₄) with 66 drops of ethanol alcohol (C₂H₅OH). Put the mixture in a worm water bath (temperature =35°C) for 15 minutes.

- Physical method by born the titanium foil chip for 8 hours in 900°C and crash it to become powder.

**Silver nanoparticles (AgNPs)**

The silver (Ag) prepared by took half kilogram of green apple. We squeezed apple and mixed with half litre of lemon juice and (0.4 gm) of silver nitrate (AgNO₃) with (14 gm) of sodium hydroxide (NaOH). Blended all of them together and put the mixture in a tube and put it in a dark place or keep it in a black envelope, then put this mixture in a cold place like refrigerator for 24 hours.

**Titanium dioxide-Silver nitrate nanoparticles**

TiO₂-AgNO₃ (titanium dioxide-silver nitrate) prepared by physical method by born the titanium foil chip for 8 hours in 900°C and crash it to became powder in specific weight with weight of silver nitrate as it explain later in this work.

**The illness and the infection**

The number of animals used in the experiment was 65 domestic rabbits adult (male type, 11 months age and 1.5-2 kg weight) as figure (1), 15 of them used for toxicity test, 5 rabbits for each type of main bionanomaterials. The other 50 rabbits have been separated to 5 groups, each group has 10 rabbits and marked in special colour to know the drug which will give after that, and one of these group was control group.

![Figure (1): Domestic male rabbits](image-url)
This experiment shows how important it is to start with a pilot study using few animals to evaluate an experimental design for drug. So, first should be exposed 40 rabbits for the radiation, where that already have been done at Al-Tuwaithah Nuclear Research Facility using the radiant element Co-60 as shows in figure (2a) with the armoured room to exposed rabbits to radiation inside to made a damage liver as shows in figure (2b).

Table (1) displays the details of drugs’ names, the colour of rabbits, and the periods of exposure. We separated the rabbits into two groups for infection. The exposure for one group (green, blue and without colour) was 10 minutes during 30 days, 10 of them shows in third column, these the second group, they were exposed 10 minutes and the period was 60 days for each rabbit, this exposure was for the rabbits that have red colour.

**Table (1): Details of drugs, colour and period**

| Drug that used later | Colour of rabbit | Period of radiation exposure daily | Days for radiation exposure | Number of rabbit |
|---------------------|------------------|-----------------------------------|----------------------------|-----------------|
| TiO$_2$             | Green            | 10 min/day                        | 30 min/day                 | 10              |
| Ag                  | Blue             | 10 min/day                        | 30 min/day                 | 10              |
| TiO$_2$+AgNO$_3$    | Red              | 10 min/day                        | 60 min/day                 | 10              |
| black seed          | Without colour   | 10 min/day                        | 30 min/day                 | 10              |

**Toxicity protocol and the drug**

The drugs’ dose weighting by very sensitive balance, where it weight till $10^{-4}$ gm/kg, as the display in figure (3)
The toxicity of titanium dioxide (TiO$_2$)

We took 5 rabbits to test the toxicity of TiO$_2$ and gave the dose (10 ml) for them for 3 weeks. After 3 weeks there was no side effect appear on.

The drug

Mix the TiO$_2$ (0.023 gm) with (1 ml) of honey and (1 ml) of onion juice. That quantity was made to give during 30 days for each rabbit.

The dose

We took 10 rabbits and marking them in green colour. Daily, gave one drop from the drug for 5 months to return their healthiness. The check-up started after 10 days by blood test of them and blood picture after 40 days.

The toxicity of silver Ag test

We took 5 rabbits to test the toxicity of silver (Ag) and gave the doses in below way

- 3 rabbits were started with (1.5 ml) in the first day. Then gave them (3 ml) in the second day. And third day were given (4.5 ml). After one day the dose became (6 ml). in the sixth day the dose became (7.5 ml). Later one day more gave them (9 ml). The seventh day the dose reached (10 ml) and continue on latest dose for 2 weeks.
- One rabbit was given dose = (7.5 ml) for 3 weeks.
- The fifth rabbit was taken him dose equal (10 ml) for 3 weeks.

After 3 weeks there was no side effect appear on all of them, that was mean, there was not toxicity of the mixture.

The drug

We made first time drag from silver nitrate (AgNO$_3$) directly by mixing AgNO$_3$ (0.02 gm) with (1 ml) of honey and (1 ml) of onion juice. That quantity was made to give during 30 days for each rabbit.

The dose

We took 10 rabbits and marking them in blue colour. Daily, gave one drop from the drug for 5 months to return their healthiness. The check-up started after 10 days by blood test of them and blood picture after 40 days.

The toxicity of titanium dioxide (TiO$_2$-AgNO$_3$)

We took 5 rabbits to test the toxicity of TiO$_2$-AgNO$_3$ and gave the dose (10 ml) for them for 3 weeks. After 3 weeks there was no side effect appear on.
The drug
Mix the TiO₂ (0.023 gm) with AgNO₃ (0.02 gm) with (1 ml) of honey and (1 ml) of onion juice. That quantity was made to give during 30 days for each rabbit.

The dose
We took 10 rabbits and marking them in red colour. Daily, gave one drop from the drug for 2 months and 1 week to return their healthiness. The check-up started after 10 days by blood test of them and blood picture after 40 days.

The toxicity of black seed
The drug
Mix the powder of black seed (0.036 gm) with (1 ml) of honey and (1 ml) of onion juice. That quantity was made to give during 30 days for each rabbit.

The dose
We took 10 rabbits and marking them in without colour. Daily, gave one drop from the drug for 5 months to return their healthiness. The check-up started after 10 days by blood test of them and blood picture after 40 days.

The treatment
After proving the damage of liver, started to treat the rabbit as a groups that made it before the infections by radiation and depending on colour and own drugs as shown in table (1) and explaining the quantities of materials and doses in section 3.6. We took the slides with 1*3 inch dimensions and made a blood film (smear) by separated the drop of rabbit blood on the slide surface as shows in figure (4).

Figure 4: A blood film (separated the drop of rabbit blood)
Later, have been coated the film by Leishman stain -figure (5)- to test the white blood cells and red blood cells (shape and numbers ).
Figure 5: Sample of film before and after coating the film by Leishman stain

The white blood cells (WBCs) and red blood cells (RBCs) pictures show the shape and the damaging in RBCs which happen due to the damage of liver and changing in its functions. These pictures explain the treatment level during the time and the acceptably of drug according to its colour.

Results and Discussions

Blood Pictures for Nano Biomaterials

From the blood pictures in the following figures, we note that the number of white blood cells (WBC) has become normal but a large difference in red blood cells (RBC) shapes, and this is a reason for damage to the liver or kidney, where is clear the upnormal shape of red blood cells, this shape is known acanthocytes or spur cells which are formed due to liver diseases like high plasma cholesterol on RBC membrane and phospholipid abnormalities in hepatic diseases or cancer makes RBC sunflower-like[13-14]. White blood cells affect the way in which the immune system shows its reaction to various diseases and the ability of the actual immune system to fight infection, so any defect in it such as an increase or decrease or its damage may have bad consequences. The results of the white blood cells examination appear in the form of numbers and percentages, as the numbers indicate the absolute value of the number of white blood cells, while the percentages indicate the part that constitutes each type of white blood cells from the total white blood cells. The absolute value of white blood cells is between 4,300 - 10,800 (the range can vary slightly between different laboratories)[15]. High values (greater than the upper limit) of white blood cells usually indicate an infection, such as pharyngitis. The very high values may indicate leukemia caused by bone marrow. Low values (less than the minimum) indicate an immune failure and a high risk of infection and infections. Figure (6) offered the blood picture of red blood cells for control rabbit and it is clear the normal shape of RBC.

Figure 6: Red Blood Cells (RBCs) picture for control rabbit
Blood analysis is a very common laboratory test, in which a sample of blood is drawn to determine the biochemical and physiological condition of blood, which would help doctors to verify the presence of a specific disease or the development of a specific medical condition, and it also helps determine the degree of efficiency of some members in the performance of its function correctly, in addition to verifying the effectiveness of some medications used in treatment. After the drawing process comes a set of instructions that must be followed strictly for the purpose of preserving the sample from damage and preparing it to suit the quality of the test that we will do.

The following table (2) shows the results of the blood and serum analysis. The results of the analysis showed that it is normal for the control group and this indicates that the animals are in good health and can be used in the experiment.

### Table (2): Results for control group

| Items             | Control rabbit | Normal                  |
|-------------------|----------------|-------------------------|
| Body weight       | 2.5 Kg         | 2.6-3.8 Kg              |
| Liver weight      | 0.20 gm/100 gm body weight | 4-10 gm body weight     |
| WBC               | 8.4 × 10^9/µL  | 4-10 10^9/µL            |
| RBC               | 5.4H × 10^9/µL | 5.0-8.0 H 106/µL        |
| Urea              | 51 mg/dL       | 42-80 mg/dL             |
| Total protein     | 6.2 mg/dL      | 5.4-7.3 mg/dL           |
| Creatinine        | 1.4 mg/dL      | 0.5-2.2 mg/dL           |
| GOT (AST)         | 47 U/L         | 10-120 U/L              |
| GPT (ALT)         | 31 U/L         | 10-45 U/L               |
| S. ALP            | 45 U/L         | 4.0-20 U/L              |
| Glucose           | 109 mg/dL      | 80-150 mg/dL            |
| Total Cholesterol | 22 mg/dL       | 10-80 mg/dL             |
| Total Calcium     | 14.6 mg/dL     | 8.0-15.5 mg/dL          |
| Total Phosphorus  | 6.5 mg/dL      | 4.4-7.2 mg/dL           |

The type of radiation damage to tissues and/or organs of the animal depend on the radiation dose to which it is exposed. Radiation, if it exceeds certain limits, can impair the functions of tissues and/or organs and lead to severe effects such as skin redness, hair loss, radiation burns and acute radiation syndrome. The higher the amount of radiation, the higher the dose rate, the more severe the effects. The damaged cells are more likely to succeed in repairing themselves if the dose the animal receives or is exposed to over a long period of time (the dose rate decreases). But there is a possibility that long-term effects will also occur if there are errors in the process of repairing damaged cells, turning those cells into radioactive cells that are still able to divide. And this transformation may lead to cancer in animals after years or even decades. These effects do not necessarily occur, although the probability of their occurrence is directly proportional to the radiation dose.

Liver and Kidney disease or disorder was detected by measuring blood urea nitrogen levels, creatinine and measuring levels of liver enzymes for infected rabbits by using radiation (table (3)).

### Table (3): Results for infected group

| Items     | One week | Two week | Three week | Four week | Five week | Six week | Seven week | Eight week |
|-----------|----------|----------|------------|-----------|-----------|----------|------------|------------|
| Body weight | 2.5 Kg   | 2.5 Kg   | 2.5 Kg     | 2.5 Kg    | 2.5 Kg    | 2.5 Kg   | 2.5 Kg     | 2.5 Kg     |
| Liver weight | 0.20 gm/100 gm body weight | 0.22 gm/100 gm body weight | 0.21 gm/100 gm body weight | 0.17 gm/100 gm body weight | 0.25 gm/100 gm body weight | 0.19 gm/100 gm body weight | 0.20 gm/100 gm body weight | 0.23 gm/100 gm body weight |
| WBC       | 12 × 10^9/µL | 15 × 10^9/µL | 23 × 10^9/µL | 24 × 10^9/µL | 265 × 10^9/µL | 31 × 10^9/µL | 24 × 10^9/µL | 26 × 10^9/µL |
| RBC       | 901 × 10^9/µL | 111 × 10^9/µL | 141 × 10^9/µL | 131 × 10^9/µL | 140 × 10^9/µL | 121 × 10^9/µL | 214 × 10^9/µL | 2.54 × 10^9/µL |
| Urea      | 100 mg/dL   | 120 mg/dL  | 106 mg/dL   | 96 mg/dL   | 107 mg/dL  | 110 mg/dL | 121 mg/dL  | 90 mg/dL   |
| Total protein | 8 mg/dL | 10 mg/dL | 9mg/dL | 11 mg/dL | 8 mg/dL | 10 mg/dL | 12 mg/dL | 1mg/dL |
| Creatinine | 3 mg/dL | 2.8 mg/dL | 4 mg/dL | 3.2 mg/dL | 5 mg/dL | 3.6 mg/dL | 4 mg/dL | 2.9 mg/dL |
| GOT (AST) | 130 U/L | 150 U/L | 200 U/L | 190 U/L | 160 U/L | 170 U/L | 150 U/L | 1508 U/L |
| GPT (ALT) | 50 U/L | 66 U/L | 70 U/L | 90 U/L | 97 U/L | 100 U/L | 85 U/L | 90 U/L |
| S. ALP | 55 U/L | 80 U/L | 89 U/L | 95 U/L | 102 U/L | 110 U/L | 99 U/L | 104 U/L |
| Glucose | 109 mg/dL  | 132 mg/dL  | 120 mg/dL   | 106 mg/dL  | 95 mg/dL   | 86 mg/dL  | 132 mg/dL | 92 mg/dL   |
| Total Cholesterol | 100 mg/dL | 120 mg/dL | 140 mg/dL | 120 mg/dL | 90 mg/dL | 85 mg/dL | 70 mg/dL | 60 mg/dL |
| Total Calcium | 15 mg/dL | 14 mg/dL | 15 mg/dL | 15 mg/dL | 14 mg/dL | 15 mg/dL | 16 mg/dL | 15 mg/dL |
| Total Phosphorus | 7 mg/dL | 8 mg/dL | 6 mg/dL | 70 mg/dL | 56 mg/dL | 6 mg/dL | 8 mg/dL | 61 mg/dL |
After the infection was diagnosed, the animals were divided into groups, each group was given treatment within a typical protocol, and a blood test was performed during a period of 10, 20, 30 and 40 days (tables 4, 5, 6 and 7).

**Table (4): Results for Treatment group after 10 days**

| Items           | Without color rabbit | Green color rabbit | Red color rabbit | Blue color rabbit |
|-----------------|----------------------|--------------------|-----------------|------------------|
| Body weight     | 2.5 Kg               | 2.5 Kg             | 2.5 Kg          | 2.5 Kg           |
| Liver weight    | 0.19 gm/100 gm body weight | 0.20 gm/100 gm body weight | 0.23 gm/100 gm body weight | 0.24 gm/100 gm body weight |
| WBC             | 8.1 x 10^3/µL        | 8.3 x 10^3/µL      | 8.7 x 10^3/µL   | 8.3 x 10^3/µL    |
| RBC             | 5.7 x 10^3/µL        | 5.3 x 10^3/µL      | 5.5 x 10^3/µL   | 5.1 x 10^3/µL    |
| Urea            | 37 mg/dL             | 44 mg/dL           | 34 mg/dL        | 38 mg/dL         |
| Total protein   | 7.1 mg/dL            | 6.9 mg/dL          | 6.7 mg/dL       | 7.7 mg/dL        |
| Creatinine      | 2.1 mg/dL            | 1.6 mg/dL          | 1.9 mg/dL       | 2.0 mg/dL        |
| GOT (AST)       | 33 U/L               | 30 U/L             | 38 U/L          | 40 U/L           |
| GPT (ALT)       | 38 U/L               | 30 U/L             | 33 U/L          | 35 U/L           |
| S. ALP          | 72 U/L               | 69 U/L             | 64 U/L          | 68 U/L           |
| Glucose         | 86 mg/dL             | 132 mg/dL          | 92 mg/dL        | 100 mg/dL        |
| Total Cholesterol | 34 mg/dL            | 29 mg/dL           | 24 mg/dL        | 27 mg/dL         |
| Total Calcium   | 14.7 mg/dL           | 14.4 mg/dL         | 15.5 mg/dL      | 15.7 mg/dL       |
| Total Phosphorus | 5.6 mg/dL           | 6.9 mg/dL          | 7.1 mg/dL       | 8.0 mg/dL        |

**Table (5): Results for Treatment group after 20 days**

| Items           | Without color rabbit | Green color rabbit | Red color rabbit | Blue color rabbit |
|-----------------|----------------------|--------------------|-----------------|------------------|
| Body weight     | 2.5 Kg               | 2.5 Kg             | 2.5 Kg          | 2.5 Kg           |
| Liver weight    | 0.22 gm/100 gm body weight | 0.21 gm/100 gm body weight | 0.17 gm/100 gm body weight | 0.25 gm/100 gm body weight |
| WBC             | 8.6 x 10^3/µL        | 8.2 x 10^3/µL      | 8.8 x 10^3/µL   | 8.5 x 10^3/µL    |
| RBC             | 5.1 x 10^3/µL        | 5.6 x 10^3/µL      | 5.3 x 10^3/µL   | 5.4 x 10^3/µL    |
| Urea            | 48 mg/dL             | 45 mg/dL           | 42 mg/dL        | 39 mg/dL         |
| Total protein   | 6.5 mg/dL            | 6.0 mg/dL          | 6.4 mg/dL       | 6.1 mg/dL        |
| Creatinine      | 1.7 mg/dL            | 1.9 mg/dL          | 1.2 mg/dL       | 1.5 mg/dL        |
| GOT (AST)       | 42 U/L               | 45 U/L             | 38 U/L          | 37 U/L           |
| GPT (ALT)       | 31 U/L               | 33 U/L             | 36 U/L          | 32 U/L           |
| S. ALP          | 43 U/L               | 63 U/L             | 76 U/L          | 68 U/L           |
| Glucose         | 112 mg/dL            | 120 mg/dL          | 100 mg/dL       | 95 mg/dL         |
| Total Cholesterol | 26 mg/dL            | 32 mg/dL           | 36 mg/dL        | 41 mg/dL         |
| Total Calcium   | 14.9 mg/dL           | 15.2 mg/dL         | 15.0 mg/dL      | 15.4 mg/dL       |
| Total Phosphorus | 6.2 mg/dL           | 6.8 mg/dL          | 6.0 mg/dL       | 6.6 mg/dL        |

**Table (6): Results for Treatment group after 30 days**

| Items           | Without color rabbit | Green color rabbit | Red color rabbit | Blue color rabbit |
|-----------------|----------------------|--------------------|-----------------|------------------|
| Body weight     | 2.5 Kg               | 2.7 Kg             | 2.6 Kg          | 2.8 Kg           |
| Liver weight    | 0.20 gm/100 gm body weight | 0.25 gm/100 gm body weight | 0.23 gm/100 gm body weight | 0.24 gm/100 gm body weight |
| WBC             | 8.7 x 10^3/µL        | 8.9 x 10^3/µL      | 8.7 x 10^3/µL   | 8.5 x 10^3/µL    |
| RBC             | 5.3 x 10^3/µL        | 5.6 x 10^3/µL      | 5.4 x 10^3/µL   | 5.8 x 10^3/µL    |
| Urea            | 45 mg/dL             | 50 mg/dL           | 60 mg/dL        | 45 mg/dL         |
| Total protein   | 6.5 mg/dL            | 6.8 mg/dL          | 7.2 mg/dL       | 7.0 mg/dL        |
| Creatinine      | 1.5 mg/dL            | 1.4 mg/dL          | 1.1 mg/dL       | 1.7 mg/dL        |
| GOT (AST)       | 50 U/L               | 38 U/L             | 30 U/L          | 40 U/L           |
| GPT (ALT)       | 40 U/L               | 35 U/L             | 25 U/L          | 30 U/L           |
| S. ALP          | 35 U/L               | 70 U/L             | 60 U/L          | 55 U/L           |
| Glucose         | 85 mg/dL             | 130 mg/dL          | 110 mg/dL       | 140 mg/dL        |
Table (7): Results for Treatment group after 40 days

| Items               | Without color rabbit | Green color rabbit | Red color rabbit | Blue color rabbit |
|---------------------|----------------------|--------------------|------------------|-------------------|
| Body weight         | 2.5 Kg               | 2.3 Kg             | 3.1 Kg           | 3.0 Kg            |
| Liver Weight        | 0.21 gm/100 gm body weight | 0.19 gm/100 gm body weight | 0.22 gm/100 gm body weight | 0.21 gm/100 gm body weight |
| WBC                 | 8.4 x 10^3/µL        | 8.4 x 10^3/µL      | 8.5 x 10^3/µL    | 8.4 x 10^3/µL     |
| RBC                 | 5.6 x 10^3/µL        | 6.0 x 10^3/µL      | 6.0 x 10^3/µL    | 5.8 x 10^3/µL     |
| Urea                | 0.0 mg/dL            | 0.0 mg/dL          | 0.0 mg/dL        | 0.0 mg/dL         |
| Total protein       | 0.4 mg/dL            | 0.5 mg/dL          | 0.6 mg/dL        | 0.6 mg/dL         |
| Creatinine          | 1.6 mg/dL            | 1.7 mg/dL          | 1.9 mg/dL        | 2.1 mg/dL         |
| GOT (AST)           | 13 U/L               | 15 U/L             | 17 U/L           | 14 U/L            |
| GPT (ALT)           | 35 U/L               | 30 U/L             | 33 U/L           | 30 U/L            |
| S. ALP              | 55 U/L               | 60 U/L             | 75 U/L           | 80 U/L            |
| Glucose             | 120 mg/dL            | 120 mg/dL          | 125 mg/dL        | 110 mg/dL         |
| Total Cholesterol   | 0.4 mg/dL            | 0.5 mg/dL          | 0.6 mg/dL        | 0.6 mg/dL         |
| Total Calcium       | 15.1 mg/dL           | 15.5 mg/dL         | 14.7 mg/dL       | 14.8 mg/dL        |
| Total Phosphorus    | 4.4 mg/dL            | 5.7 mg/dL          | 5.9 mg/dL        | 6.7 mg/dL         |

At the 40th day, has been made a blood film (smear) to see the shape of red blood cells (RBC) as the figure (7) shows, where the part (a) offers the RBC of the group that given TiO2 drug after 40 days, and part (b) offers the RBC of the group that given TiO2 drug after 98 and part (c) offered RBC after 155 days (5 months).
Figure (7): Image is a film of blood after a- 40 days, b- 98 days and c- 155 days of giving (TiO₂) drug and the number of white blood cells.

At the 40th day, has been made a blood film (smear) to see the shape of red blood cells (RBC) as the figure (8) shows, where the part (a) offers the RBC of the group that given AgNO₃ drug after 40 days, later started given AgNPs drug in 70th day, and part (b) offers the RBC of the group that given TiO₂ drug after 98 and part (c) offered RBC after 155 days (5 months).
Material: AgNO₃
Date: 1/11/2019
WBCs: 32000 cells per microliter

Material: Ag
Date: 28/12/2019
WBCs: 5000 cells per microliter
Figure (8): Image is a film of blood after a- 40 days, b- 98 days and c- 155 days of giving (AgNO3 and after 70 days Ag) drug, and the number of white blood cells.

At the 40th day, has been made a blood film (smear) to see the shape of red blood cells (RBC) as the figure (9) shows, where the part (a) offers the RBC of the group that given TiO2-AgNO3 drug after 40 days, and part (b) offers the RBC of the group that given TiO2-AgNO3 drug after 67 days (2 months and one week).
Figure (9): Image is a film of blood after a- 40 days and b- 67 days of giving (TiO\textsubscript{2}-AgNO\textsubscript{3}) drug and the number of white blood cells

At the 40\textsuperscript{th} day, has been made a blood film (smear) to see the shape of red blood cells (RBC) as the figure (10) shows, where the part (a) offers the RBC of the group that given black seed drug after 40 days, and part (b) offers the RBC of the group that given black seed drug after 98 and part (c) offered RBC after 155 days (5 months).
Figure (10): Image is a film of blood after a- 40 days, b- 98 days and c- 155 days of giving black seed drug and the number of white blood cells.

Conclusions

1. Synthesize bio-nanostructure from different natural and chemical resources like TiO$_2$, Ag and herbs and then characterize using various techniques.
2. Measure the toxicity of the produced nanomaterial’s post the characterization

References

1. Small, E.; National Research Council Canada (2006). Culinary Herbs. NRC Research Press. p. 1. ISBN 978-0-660-19073-0. Retrieved 9 October 2018.
2. Patrick Curry: "Culpeper, Nicholas (1616–1654)", Oxford Dictionary of National Biography (Oxford, UK: OUP, 2004)
3. Wrensch, Ruth D. (1992). The Essence of Herbs. University Press of Mississippi. p. 9.
4. ^Jump up to: a b Tapsell LC, Hemphill I, Cobiac L, Sullivan DR, Fenech M, Patch CS, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE (2006). "Health benefits of herbs and spices: The past, the present, the future". Medical Journal of Australia. 185 (4): S1–S24.

5. ^Adele G Dawson (2000). Herbs, Partners in Life: Healing, Gardening and Cooking with Wild Plants. Bear & Co. pp. 5–6.

6. ^Dillehay T, Rossen J, Ugent D, Karathanasis A, Vásquez V, Netherly P (2010). "Early Holocene coca chewing in northern Peru". Antiquity. 84 (326): 939–953. doi:10.1017/S0003598X00067004.

7. ^Ernest Abel (1980). Marihuana: The First Twelve Thousand Years (PDF). New York: Springer. ISBN 978-0-306-40496-2. Retrieved 2018-07-25.

8. ^Cooper, Guy; Taylor, Gordon I. (1986). English Herb Garden. Random House.

9. ^Panda, H. (2015). Herbal Cosmetics Handbook (3rd ed.). Asia-Pacific Business Press.

10. John R Burnett, Amanda J Hooper, Vitamin E and Oxidative Stress in Abetalipoproteinemia and Familial Hypobetalipoproteinemia, 2015, pages:59-62.

11. Mary M. Christopher, Michelle G. Hawkins, Andrew G. Burton, Poikilocytosis in Rabbits: Prevalence, Type, and Association with Disease, 2014, pone.0112455.

12. Aelred D. Geis, Normal Blood Cell Counts for the Cottontail Rabbit, 1957, Page 136.

13. John R Burnett, Amanda J Hooper, Vitamin E and Oxidative Stress in Abetalipoproteinemia and Familial Hypobetalipoproteinemia, 2015, pages:59-62.

14. Mary M. Christopher, Michelle G. Hawkins, Andrew G. Burton, Poikilocytosis in Rabbits: Prevalence, Type, and Association with Disease, 2014, pone.0112455.

15. Aelred D. Geis, Normal Blood Cell Counts for the Cottontail Rabbit, 1957, Page 136.