ORIGINAL ARTICLE

Evaluation and acute toxicity studies of herbally prepared silver nanoparticles and Rajat Bhasma using modern analytical instruments

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Abstract

Background: Rajat Bhasma (RB) is a herbomineral formulation used in Ayurvedic practice. The traditional system of medicine expresses that bhasma contains various metals and minerals, used in different disease conditions but due to the presence of heavy metals, and is questioned for their safety aspect by regulatory authorities. Phytotherapeutics require a scientific approach to deliver the components in a sustained manner to increase the patient compliance and avoid repeated administration. **Aim:** To study comparative evaluation of herbally prepared silver nanoparticles and Rajat Bhasma using modern analytical Instruments and acute toxicity study. **Materials and Methods:** The ginger rhizomes (*Zingiber officinale*) and fenugreek seed (*Trigonella foenum-graecum*) extract were selected due to its antidiabetic activity and silver in the form of RB is recommended for antidiabetic activity in Ayurveda. The crude extract was prepared by maceration method and synthesis of silver nanoparticles was done by adding dropwise extract of herbal in silver nitrate solution. Further, acute oral toxicity was performed on Wistar albino rat. **Results and Discussion:** Herbal silver nanoparticles (HSNs) were comparatively evaluated with RB using modern analytical technics. Acute toxicity study revealed the safety of HSN over the RB. **Conclusion:** Integrating nanoscience as a novel drug delivery system (NDDS) in traditional medicine enriches the potential of herbal drugs for treating chronic diseases. In the present study, HSNs were prepared using ginger rhizomes (*Z. officinale*) and fenugreek seeds (*T. foenum-graecum*) extracts and were compared with marketed RB by employing acute toxicity studies and characterization was done using ultraviolet spectroscopy, Fourier transform infrared, particle size analyzer, X-ray diffraction, and scanning electron microscopy techniques. The results of the study showed that HSN can be an alternative for herbomineral formulations.

Key words: Rajat Bhasma, herbal silver nanoparticles, herbomineral formulation

INTRODUCTION

The Ayurvedic formulation contains different types of metals and minerals, which are passed through many traditional processes and finally transformed into a suitable therapeutically active dosage form for various diseases.[1] This formulation prepared using various traditionally reported and scientifically approved methods such as Shodhana, Marana, and Bhasmikarana. Rasashastra is a science, dealing mainly with the processing and therapeutic utilization of metals, and minerals.[2] Processed metals were used very frequently by seers of the Indian tradition in different disease conditions with great authority.[3] They obtained these preparations after exposing the metals through a range of physicochemical processes in the presence of various medicinal herbs. When such metals and minerals undergo processes of purification and calcination, they result in formation fine powders termed bhasma. The Ayurveda such as Sushruta, Acharya Charaka, and Vaghbata knew the importance of Bhasma

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(calcined formulation) which contains different types of metals such as Cu, Ag, Au, Fe, Zn, and Hg where these are associated with organic macromolecules derived from the herbal extract making these biologically assimilable.[4] These powders are also termed herbometallic preparations as they contain both metallic and herbal ingredients.[5] The matter related to the usage of metal- and mineral-based preparations for the therapeutic purpose is among one of the most controversial issues of the present day. Ayurvedic classics reported that they are potent drugs and should be used judiciously. Generally metals and minerals are purified due to their toxic nature but metallic compounds such as sulphides are not toxic in therapeutic doses.[6] The curiosity about the metal- and mineral-based preparations in Ayurvedic classics and other traditional systems of medicine is safe as well as effective but them having limited therapeutic benefits. There are non-regulated standard operative procedures for the manufacturing of these drugs. Furthermore, there is a lack of standardization in all aspects. Many a time, modernization of the technique is undertaken without assessing the impact of such changes on the safety and efficacy of the final product. Due to these Aquinas by manufacturers, Ayurvedic formulations are banned in most of the countries in Europe.[7] The major benefits of the nanomedicines in the drug delivery system. This shows a rapid mechanism of action; recently, using these novel approaches, various drugs are under investigation such as anticancer agents. Another advantage of nanotechnology is that it reduces toxicity and side effects of drugs. The method of preparation of RB is tedious and time consuming involving the strenuous process of cleansing and refinement, generally termed as purification. The evaluation of efficacy and quantity of the bhasma is performed by utilizing complementary analytical techniques that eventually lead to morphological, structural, elemental, and chemical characterization of the sample. The present study is an attempt to suggest the beneficial properties of HSN which can be an alternative over traditional metallic preparations in terms of delivering desired therapeutic efficacy in low dose, safety and can be analytically standardized for regulatory compliance of different authorities and thus improving their acceptability around the globe.

MATERIALS AND METHODS

Collection of Materials

Ginger rhizomes (*Zingiber officinale*) and fenugreek seeds (*Trigonella foenum-graecum*) were purchased from local market of Pimpri, Pune, in the month of August 2017. Ginger rhizomes (*Z. officinale*) and fenugreek seeds (*T. foenum-graecum*) were washed thoroughly with distilled water to remove the dust on the periphery of the seed. Cleaned rhizomes and seeds were dried and removed, transfer to electric grinder to make powder.

Preparation of Ginger Rhizomes and Fenugreek Seeds Extract

The powdered drugs were weighed about 200 g and homogenized using mechanical stirrer (Remi Motors) for 1 h in separate 1000 ml beaker containing 500 ml distilled water. The mixture was heated for 15 min, cooled, and was filtered through soft muslin cloth. The extract was stored at 4°C for further use.

Synthesis of Herbal Silver Nanoparticles (HSNs)[8,9]

One millimolar solution of silver nitrate ($AgNO_3$) was prepared in two different conical flasks and used for the synthesis of silver nanoparticles. Each flask contains 100 ml of 1 mM solution of silver nitrate (Ag+) was heated on a magnetic stirrer at 1200 rpm (Remi Motors). The dropwise addition of previously prepared ginger extract to the silver nitrate solution. The synthesis of silver nanoparticles was carried out at 60–70°C temperature until the endpoint is observed. On adding the light reddish-brown color ginger extract to colorless silver nitrate solution, the formation of AgNPs occurred and they exhibited a color change due to surface plasmon resonance. The intensity of color was directly proportional to the formation of AgNPs. The color change was very rapid and as soon as the two solutions were mixed, the solution turned brown and within 10 min, it turned to dark brown and by 2 h, the solution turned black. The color change in the reaction mixture such as silver metal solution + herbal extract was recorded through visual observations.

Analysis of Herbomineral Formulation and HSNs[10-12]

Ultraviolet (UV)–visible spectrophotometer for silver nanoparticles

The UV spectra of silver nanoparticles were taken on UV-1700 (Shimadzu Instruments, Japan). Spectra were scanned from 350 to 700 nm wavelengths. A plasmon absorption band, characteristic of ginger silver nanoparticles and fenugreek silver nanoparticles, was observed at 425 nm, respectively [Figure 1].

Fourier-transform infrared (FT-IR) spectroscopy

Herbomineral formulation, ginger AgNPs and fenugreek AgNPs taken 5 mg each and 100 mg KBr, was added to each formulation and triturated in mortar pestle and placed in the sample cells and spectra run from 4000 to 400 cm^{-1}.

Particle size and zeta analyzer

The particle size and zeta of herbomineral formulation (Rajat Bhasma [RB]) and HSNs were determined using Malvern (version.2) instrument at AISSMS College of Pharmacy, Pune.

X-ray diffraction (XRD)

The XRD powder pattern of Herbomineral formulation (Rajat Bhasma) and herbal silver nanoparticles were recorded on mini–X-Ray Diffractometer (Miniflex, Rikagu Tokyo, Japan) by using nickel filter over the range 20.0º – 80.0° at BG ACS College, Sanghavi, Pune.

Scanning electron microscopy (SEM)

The shape and surface characterization were investigated using SEM (JEOL-6000, USA) at Physics Department, Savitribai Phule Pune University, Pune.

Sample preparation for SEM

About 10 mg sample was stuck on carbon double adhesive tape on the metal dye. Then, sample was placed in coater machine for coating of platinum (JFC-1600 auto fine coater) using 15 lbs pressure for 10 min. After coating, the sample was placed in SEM for the characterization.

Oral Acute Toxicity study of Herbomineral Formulation (RB) and HSNs[13,14]

Wistar albino female rat (150–200 g) was housed in standard (28 cm × 21 cm × 14 cm size cage with three animals) opaque polypropylene cage provided with dust free paddy husk as bedding material, and was maintained on a photoperiod 12 h light/dark cycle in registered animal house (198/PO/Re/S/2000/ Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA]) of Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018. Feed and water were provided *ad libitum*. The room temperature was maintained at approximately 22 ± 5°C with relative humidity (50–70%). All experimental test paradigms were approved (Protocol No. DYPIPSR/"Institutional Animal Ethics Committee" [IAEC]/17-18/P-30) by the IAEC and were conducted in adherence to the guidelines stipulated by the CPCSEA, Animal Welfare Division, Ministry of Environmental and Forests, Government of India, India. The acute oral toxicity

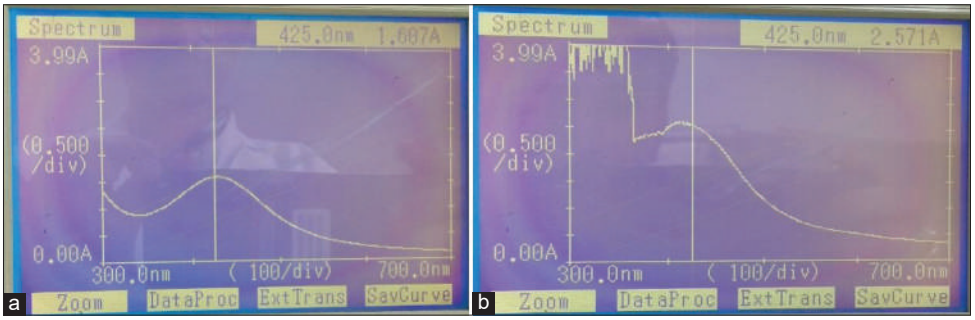

Figure 1: Ultraviolet–visible spectra of ginger nanoparticles (a) and fenugreek nanoparticles (b)

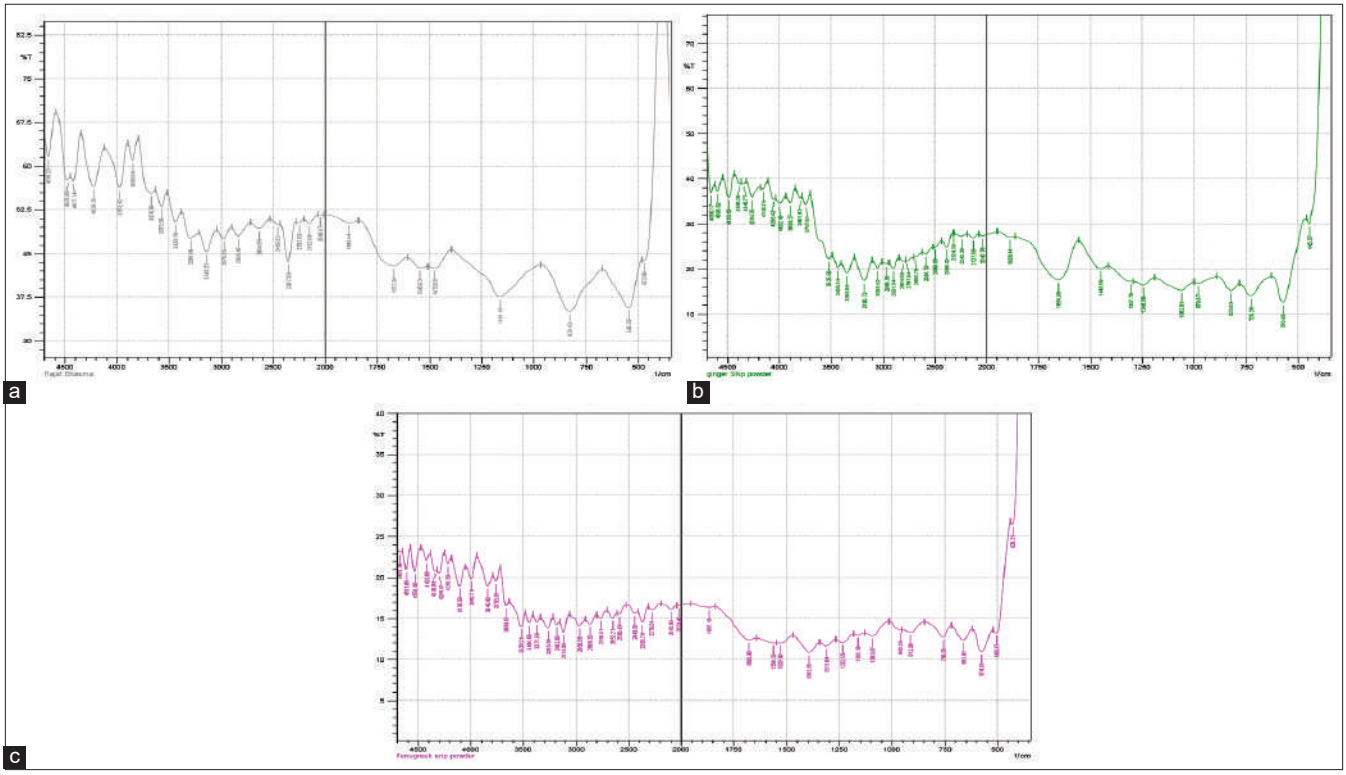

Figure 2: Infrared spectra of Rajat Bhasma (a), ginger nanoparticles (b), fenugreek nanoparticles (c)

of the RB, ginger nanoparticles, and fenugreek nanoparticles was investigated according to the OECD 423 guideline for Testing of Acute Oral Toxicity-Acute Toxic Class Method (OECD guidelines, 2001). After 7-day adaption to laboratory conditions, three rats (female) were administered with the RB, three rats (female) were administered with the ginger nanoparticles, and three rats (female) were administered with the fenugreek nanoparticles in a single dose by oral gavages. The RB was administered to the animals, p.o. at dose level of 2000 mg/kg in 0.5% carboxymethyl cellulose with <1.5 ml volume according to OECD guideline No. 420. The ginger nanoparticle and fenugreek nanoparticle were dissolved in 1.5 ml water.

Histopathology studies

The animals were anesthetized with anesthetic ether. Liver, kidney, lungs, and tissue were isolated. The isolated tissue was trimmed into small pieces and preserved in 10% formalin for 24 h and were submitted to Chaitanya Laboratories for histopathological studies. The tissues were stained with hematoxylin-eosin stain as per the standard procedure described by Luna.

RESULTS

The prepared HSN was evaluated on qualitative and quantitative aspects and the report shows satisfactory results. The FTIR spectra show some organic and inorganic compounds present in the formulation in Figure 2, (1). Ginger-3435 cm−1 -NH stretch, 3053 cm−1 _Aromatic CH stretch, 1655 cm−1 -carbonyl, (2). Fenugreek- 3444 cm−1 -NH stretch, 2986 cm−1 -CH3 stretch, 1680 cm−1 _carbonyl, 1564–1529 cm−1 _Aromatic C=C stretch. The particle size of RB, ginger AgNPs, and

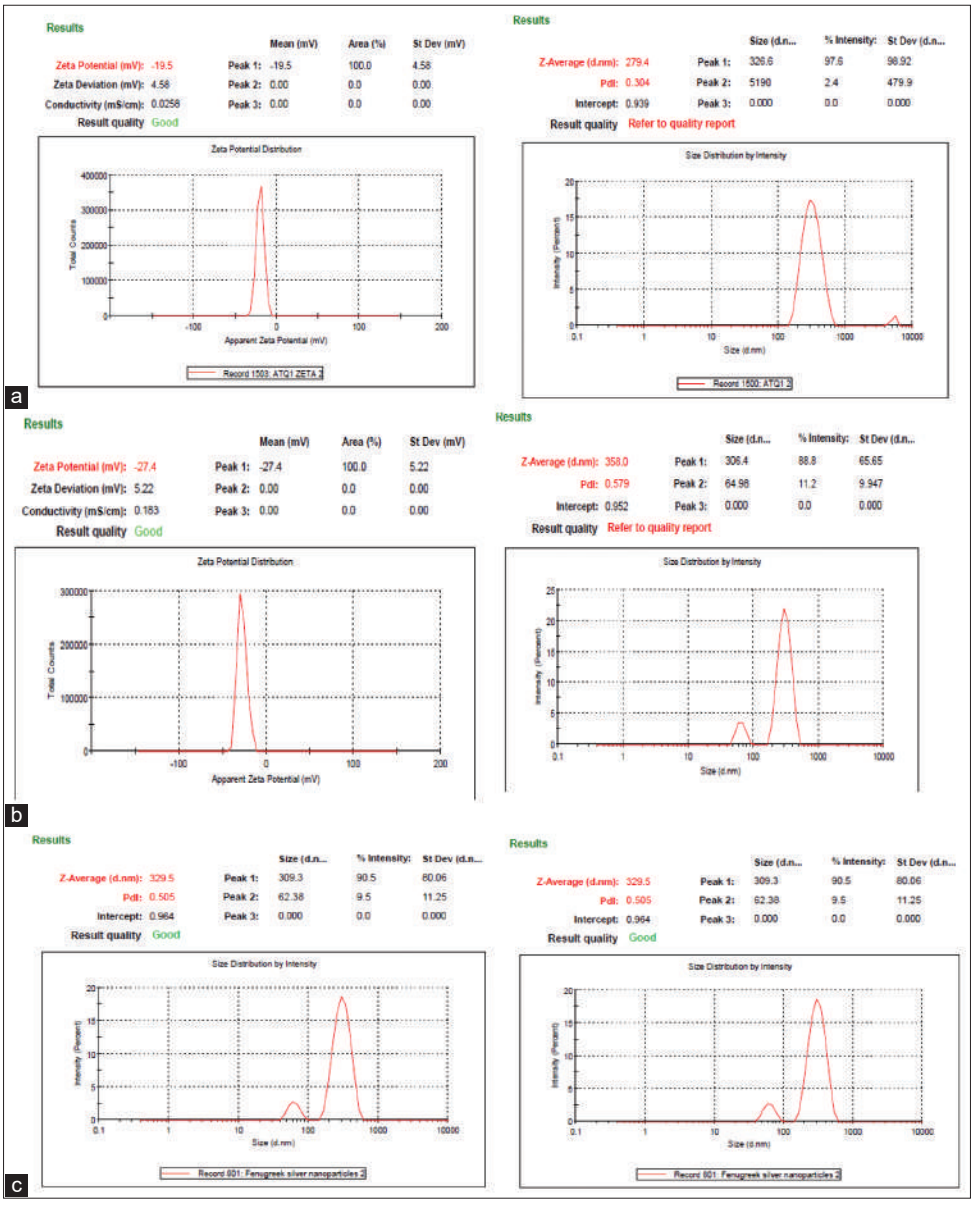

Figure 3: Particle size and zeta potential of Rajat Bhasma (a), ginger nanoparticle (b), and fenugreek nanoparticle (c)

fenugreek AgNPs was found 279.4 nm, 358.0 nm, and 329.5 nm, respectively. Zeta potential was found −19.5 mV, −27.5 mV, and −25.2 mV, respectively [Figure 3]. The XRD pattern of RB shows more crystalline particles and shows the luster on a 2θ°scale, it was found 35° with an intensity of peak mounting to 650, 2θ° for ginger AgNPs and fenugreek AgNPs was found 33° and 34°, respectively [Figure 4]. The shape and surface characterization of RB were estimated using SEM and found more spherical [Figure 5]. The group of rats which were given acute dose of RB as a single oral dose at 2000 mg/kg each, respectively, produced mortality, whereas the other group of rats was given acute dose of ginger AgNPs and fenugreek AgNPs as a single oral dose at 2000 mg/kg each, respectively, did not produced any mortality nor altered the behavior patterns of the rat during the observation period of 14 days. Therefore, the ginger nanoparticle and fenugreek nanoparticle were found to be safe at 2000 mg/kg dose. The histopathology study for these groups was performed on lungs, liver, and kidney of rats which showed normal histology in the groups treated with ginger AgNPs [Figure 6] and fenugreek AgNPs [Figure 7] which were comparable with control group [Figure 8].

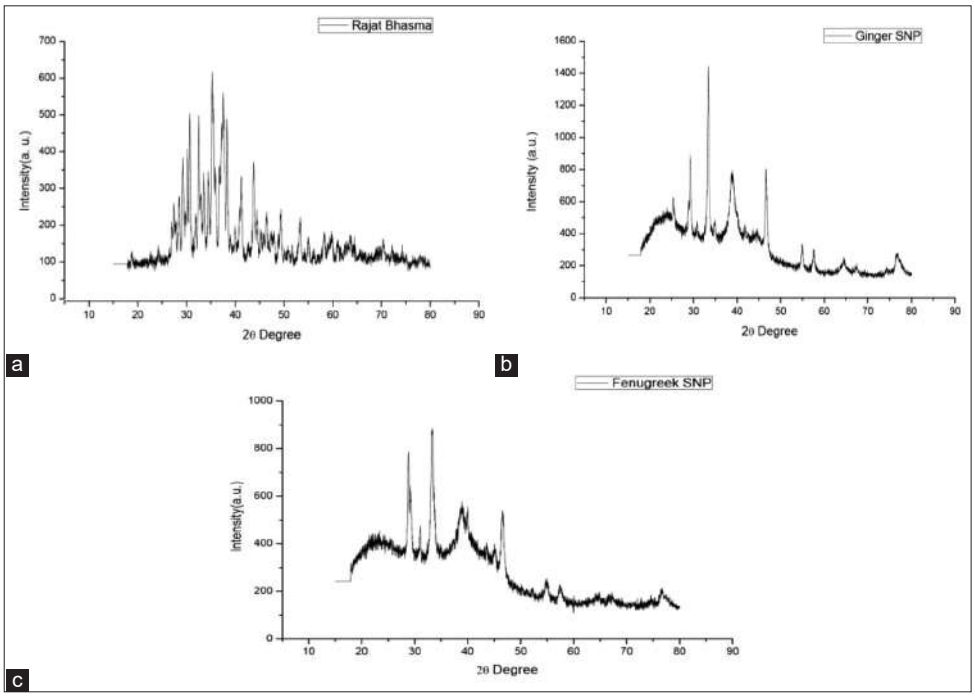

Figure 4: X-ray diffraction of Rajat Bhasma (a), ginger nanoparticle (b), and fenugreek nanoparticle (c)

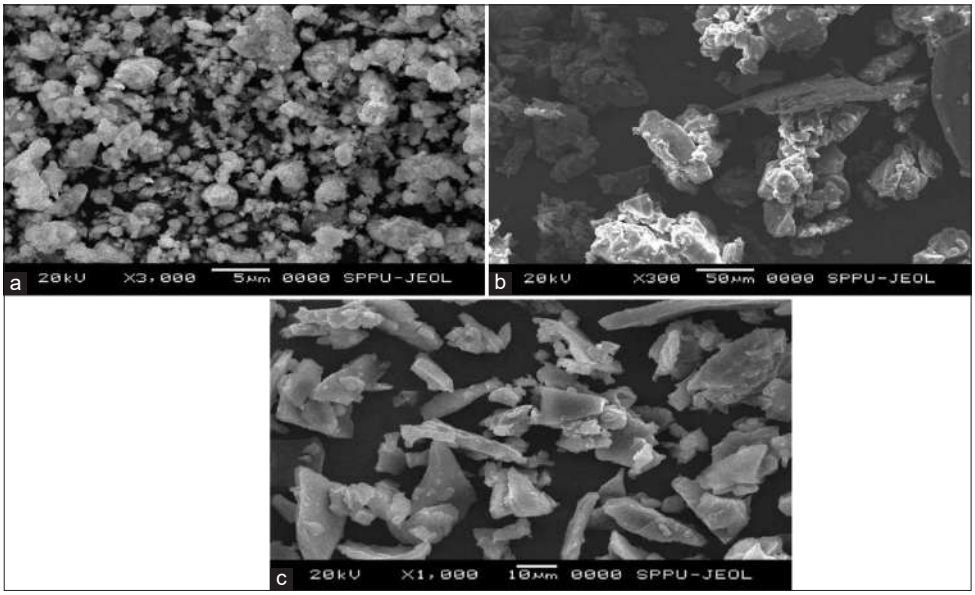

Figure 5: Scanning electron microscopy of Rajat Bhasma (a), ginger nanoparticles (b), fenugreek nanoparticles (c)

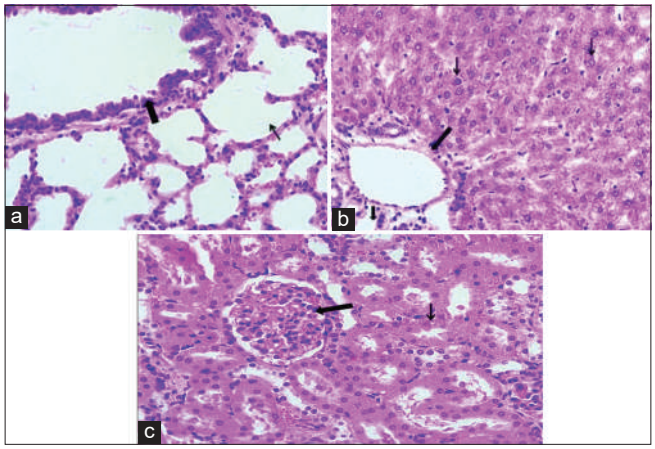

Figure 6: Histopathology of ginger AgNPs treated group lungs (a), liver (b), and kidney (c)

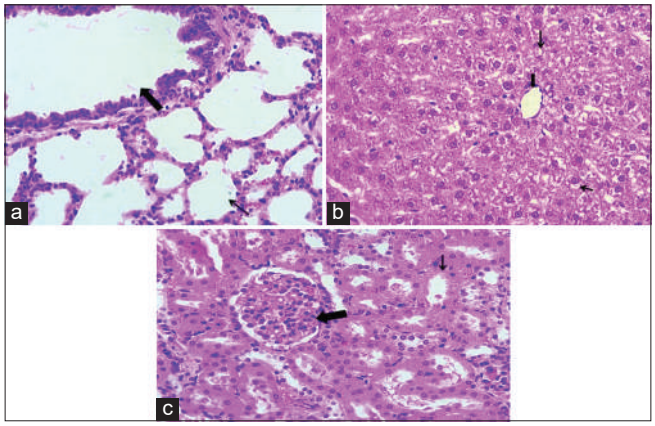

Figure 7: Histopathology of fenugreek AgNPs treated group lungs (a), liver (b), and kidney (c)

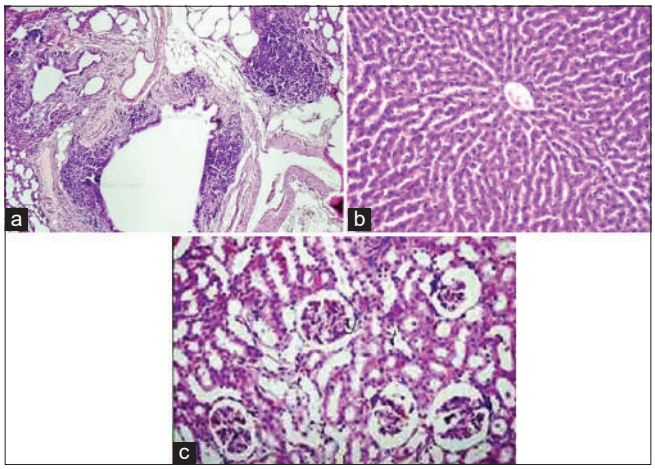

Figure 8: Histopathology normal control group lungs (a), liver (b), and kidney (c)

DISCUSSION

Nanotechnology has been the focus of considerable attention in medicine due to its nanostructure and easily interacting with the body. Pharmacokinetics and biodistribution of active ingredients can be improved remarkably with nanodrug delivery systems by targeting them to the specified site, thereby efficacy and bioavailability can be improved and drug toxicity will be reduced. The marketed RB was found toxic whereas the in-house prepared herbal nanoparticles found non-toxic and safe at the same dose. The above study revealed that HSN can be a good alternative for such metal-based Ayurvedic formulation. The marketed herbomineral formulations need to be standardized using advance analytical instruments and animal toxicity studies before marketing. Moreover, the manufacturing methods of herbal nanoparticles take less time as compared to the traditional process and, hence, are economic with a better safety profile.

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