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RESEARCH PAPER

Nanohydroxyapatite synthesized from kombucha SCOBY and its effect on ovariectomized-induced osteoporosis in rats

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ABSTRACT

Objective(s): Calcium phosphates, particularly hydroxyapatite, are the main inorganic compounds of vertebrate bone.In this study, nanohydroxyapatite was prepared using kombucha Symbiotic Culture of Bacteria and Yeast (SCOBY), and its effect was investigated on osteoporosis in ovariectomized rats.

Materials and Methods: Kombucha-nanohydroxyapatite was synthesized by adding phosphoric acid to the kambucha scoby. Characterization of the nanoparticle was performed through X-ray diffraction, X-ray fluorescence, and transmission electron microscopy techniques. Female rats were divided into 5 groups: control, ovariectomized groups, and three ovariectomized groups treated with concentrations of 25, 50, and 100 mg/kg of nanoparticles. At the end of the treatment period, the levels of calcium, phosphorus, parathyroid hormone, and activity of alkaline phosphatase were measured in the blood samples. Calcium and phosphorus levels were also measured in bone and liver. The bone was evaluated histopathologically.

Results: The synthesis of nanohydroxyapatite with particle size of 30 nm was confirmed through the use of X-ray diffraction (XRD) and TEM techniques. A significant increase in calcium and phosphorus levels in the femur bone was observed in the ovariectomized group, which received the highest nanoparticle concentration compared to the ovariectomized group. Parathyroid hormone and alkaline phosphatase activity inhibition were increased in ovariectomized rats following treatment with the highest nanoparticle concentration. In the mentioned group, bone trabeculae proliferation and increased lacuna-containing osteocytes were also observed.

Conclusion: This study suggested that the highest concentration of kombucha-nanohydroxyapatite could be partially absorbed into bone tissues and recover the bone-destructive changes caused by ovariectomy, although additional experiments are needed for confirmation.

Keywords: *Kombucha SCOBY, Nanohydroxyapatite, Osteoporosis, Ovariectomy, Rat*

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INTRODUCTION

According to recent international statistics, osteoporosis will lead to fractures in 1 out of every 3 women and 1 out of every 5 men over the age of 50 [1].

Osteoporosis is described as a slowly developing and asymptomatic condition that is often not diagnosed until a fracture happens. Fractures commonly occur at the hip, wrist, vertebrae, and pelvis. This process is often labeled as “silent pain” due to its slow progression and lack of noticeable symptoms. In the advanced stage of the disease, the bones may become so weak that a fracture occurs with minor or spontaneous stress [2]. Osteoporosis is mainly caused by the decrease in estrogen and sex androgens after menopause, leading to decreased absorption of bone mineral nutrients, especially calcium [3].

Various research has suggested that consuming calcium as an adjunctive therapy could be a beneficial way to help stop the development of osteoporosis [4,5]. The main and essential elements found in bones and teeth are calcium and phosphorus, which are stored as hydroxyapatite crystals [6]. Hydroxyapatite, a mineral characterized by a hexagonal structure and comprising calcium and phosphate groups, has a general chemical formula of Ca10 (PO4)6(OH) 2. It is a bioactive non-toxic and non-immunigenic

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material [2, 7]. In recent times, there has been a notable focus among researchers on the synthesis and utilization of synthetic of hydroxyapatites, stemming from their structural resemblance to bone and exceptional biocompatibility. Making synthetic hydroxyapatites can be done using inexpensive and organic resources [8, 9].

Kombucha, a fermented drink, has been around for over 2,500 years and is historically important to the Manchurian people.The beverage boasts a rich flavor profile, merging sweetness and tartness. Kombucha gained widespread popularity in China and Japan after it was introduced. The symbiotic interaction between eukaryotic microorganisms, such as yeasts, and prokaryotic microorganisms, specifically bacteria, in a conducive environment, such as sweet tea, serves as the foundation for the production of kombucha beverage. This procedure leads to the creation of several advantageous substances by the microorganisms [9, 10]. The symbiotic community of bacteria and yeast (SCOBY) which formed in kombucha is variable in size and amount of each microorganism in different kombucha formulations [11]. It is suggested that the natural microorganisms in kombucha, along with its polymeric compounds, could be useful for creating hydroxyapatite nanoparticles. On the other hand, the kombucha SCOBY is often thrown away, but its ability to produce nanohydroxyapatite is much more economical than many other organic substances. Therefore, in the present study, after determination of the compounds in scoby, the possibility of using it during the production of nanohydroxyapatite was tested and then the effect of the produced nanoparticles on the prevention of osteoporosis in ovariectomized rats was investigated.

MATERIALS AND METHODS

Nanohydroxyapatite kombucha SCOBY synthesis

First, kombucha drink was prepared in shallow wide glass container by using 15 g black tea which was added to 750 ml boiled distilled water. Then, 50 g white sugar was added to each container and after complete dissolution, a piece of kombucha SCOBY was added to each container as the fermentation initiator [12, 13]. Following this, a clean linen cloth was used to seal the lid of the container, and the vessel was put in a dark place with a temperature of 27 °C to enable the kombucha SCOBY to grow to its maximum size. In the next step, the SCOBY were separated and after drying in the sunlight, SCOBY were cut into pieces and then dried in a microwave oven (Model MS95CR-GSC) at 322 °C for 10 min. Then, the dried SCOBY was powdered in a mortar. The resulting powder was placed in an oven at 322 °C for 5 days every day for 2 hr until turning white. Following this, the powder that had been accumulated underwent a three-step cleansing process using deionized water, with each repetition of the cleansing process filtering the water through filter paper.The collected powder on the filter paper was subsequently desiccated in an oven at 90 °C. After adjusting pH of the mixture to 8.5; it was centrifuged (5000 rpm, 15 min) and then was dried in an oven for 24 hr at 37 °C.

Characterization

The X-ray fluorescence (XRF) apparatus (model XD-8010) was used for detection of the type and the amounts of elements in scoby powder nanoparticles. The X-ray diffraction (XRD) apparatus (model EMMA) was used for investigation of the X-ray diffraction pattern and measurement of the crystalline structure and relative size of the particles in the synthetized nanohydroxyapatite kombucha. The technique of transmission electron microscopy (TEM) was employed to examine the diameter of the synthesized nanoparticles.

Animals and grouping

The total number of 30 female rats (230-160 g) were delivered from the animal nest of Falavarjan Branch, Islamic Azad University, Isfahan. The rats were grouped as follows: A) Ovariectomized group in which the ovaries of rats were removed from the body at the beginning of the test. Ovariectomy was done after anesthetizing the rats by injecting 1 ml of 10% ketamine and 2% xylazine mixture. Briefly, the fallopian tube was pulled out, a surgical thread was tied around the ovary, and following ovary cut, the contents back in and then the wound was sutured. The ovariectomized rats were received water and food only for three months in order to induce osteoporosis [14]. B, C&D): Three groups of ovariectomized rats (n = 6 in each group) which treated with three concentrations of nanohydroxyapanite (25 mg/kg, 50 mg/kg, and 100 mg/kg) while receiving water and food similar to the previous group. The treatments were done after three months keeping in normal conditions, through intraperitoneal injection of 1 ml nanohydroxyapanite for 4 weeks, twice a week.

E): The control group which did not undergo an ovariectomy. This group only underwent surgery to induce stress and their abdomen was sutured. To each rat in this group, 1 ml physiological serum was injected each time similar to the treatment groups.

Measurement of factors in serum

At the end of rats' treatment period, each rat was weighed and then a blood sample was taken directly from the heart. Then, 2 ml of blood was transferred into tubes without anticoagulant and was centrifuged at 4000 rpm for 5 min in order to separate the serum, and the levels of phosphorus, calcium and parathormone hormone (PTH) as well as the activity of ALP was measured in the serum samples by using experimental kits (Pars Azmun, Iran) according to the manufacturer instructions.

Determination of the serum phosphorus and calcium concentration

In accordance with the protocol outlined in the kit, a volume of 200 µl of a reagent consisting of a mixture of glycine in ammonium molybdenum buffer and glycine in sulfuric acid buffer at a ratio of 1:4 was introduced to three microliters of blood serum. Subsequently, after duration of five minutes had elapsed, the absorption of the resulting solution was measured at a wavelength of 340 nm using an ELISA Reader. After a 5 min incubation period, the measurement and meticulous recording of light absorbance at a 630 nm wavelength was conducted. The light absorbance at a wavelength of 630 nm was meticulously measured and recorded after an incubation period lasting 5 min.

Measurement of alkaline phosphatase activity

Initially, a solution containing 1 mmol/L of diethanolamine was combined with a solution containing 0.5 mmol/L of chloride (referred to as Reagent 1), along with another solution containing 10 mmol/L of p-nitrophenyl phosphate (referred to as Reagent 2). Subsequently, a quantity of 200 ml of the aforementioned solution was introduced to 4 ml of serum, followed by a 5 min incubation period. The measurement of optical absorption at 405 nm was carried out and properly recorded during this period.

Measurement of PTH

Samples, calibrators, and controls were taken out from the wells in the designated amount, and the unused wells were put back into the aluminum bag. Following that, the cover was secured, and the complete equipment was conscientiously kept in a cold environment. The wells were filled with either a sample, calibrator, or control, amounting to 25 µL. A volume of 50 µL of PTH biotin conjugate solution was introduced into each well. It should be emphasized that all remedies were administered close to the well's termination. In the given experimental protocol, a volume of 50 µL of atrium solution, conjugated with PTH, was meticulously dispensed into each individual well. The plate was subjected to incubation for a duration of 90 min at ambient temperature conditions, utilizing an ELISA shaker with rotational speeds ranging from 500 to 600 revolutions per minute (RPM). Subsequently, the substances contained within the plate were appropriately dispensed. A volume of 300 µL of the diluted washing solution was introduced into each well and subsequently removed through draining. The washing procedure was repeated thrice more, resulting in a total of four wash cycles. A volume of 100 µL of substrate solution was dispensed into all wells, followed by incubation of the plate for a duration of 15 min at ambient room temperature. A volume of 50 µL of Stop solution was introduced into each well, followed by gentle manual shaking of the plate on the table's surface for a duration of 20 seconds. Subsequently, the contents of the plate were analyzed using an ELISA device, with measurements performed at a wavelength of 450 nm while utilizing a reference filter of 630 nm. The results obtained were properly documented. The mentioned test was carried out using the Monobind ELISA PTH kit produced by the Monokit company, which is worth noting.

All steps of working on animals were done according to ethical principles and the relevant license was obtained with the code IR.IAU.FALA.REC.1398.002 from the animal ethics committee of Falavarjan branch, Islamic Azad University, Isfahan, Iran.

Histological examination of tissues

After killing the rats according to the principles of ethics of working with laboratory animals, the skin was split and after removing the thigh muscles, the femur was completely and accurately separated from the joints. Other tissues including liver, spleen, kidney, and lung also removed and all tissues were placed in formalin 10%. Bone

decalcification was performed using 5% to 10% buffered formic acid for 10 days. In the next step, liver, kidney, lung, spleen, and bone samples were placed in 10% formalin for 24 hr for fixation. Finally, the samples were dehydrated through a 70, 80, 90, 95, and 100% ethanol series. Samples were then treated with molten paraffin wax. Paraffin blocks were made of tissues and 5µm slices were prepared by microtome. The tissue sections were stained with hematoxylin and eosin (H & E) [15].

Measurement of calcium and phosphorus levels in tissues

Perchloric acid and nitric acid in a ratio of 1 to 4 were added to liver and bone tissues (1mg of each sample), and kept until complete digestion. Then, the mixtures were homogenized thoroughly by stirring with a glass mixer. The samples were later transferred to a steam bath and after removing the excess acid, the amount of phosphorus and calcium in the tissues were measured spectrophotometrically by inductively coupled plasma mass spectrometry (ICP) apparatus [16].

Data analysis

Data were collected as mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA) by SPSS statistical software. Significance levels were considered as P≤ 0.05, P≤ 0.01 and P≤ 0.001.

RESULTS

According to the XRF analysis conducted on the kombucha SCOBY (Table 1), The results showed that about 60 % calcium oxide in the sample and this scoby is good potential for hydroxyapatite synthesis.

Particle size determination

The X-ray diffraction of the samples was conducted and compared with the JCPDS (Joint Committee on Powder Diffraction Standards) card No. 9-432 for hydroxyapatite. The obtained results are presented in Fig. 1. The following analysis illustrates the production of hydroxyapatite nanoparticles.The mean size of the particles was determined by Debye-Scherer formula [17.18]. It was found less than 30nm for sample.

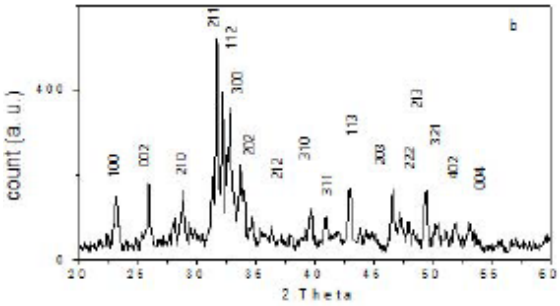

Fig. 1. XRD patterns of the Nanohydroxyapatite

Fig. 2 illustrates the TEM photograph of the hydroxyapatite sample. It can be seen that, there is a uniform size distribution approximately in whole of the photograph. It means that the synthesis manner has been suitable. The size of the particles was determined around 30 nm from TEM photograph.

Analysis of serum and tissue factors

According to the information provided in Table 2, it can be seen that the alkaline phosphatase activity was significantly increased after the ovariectomy procedure, when

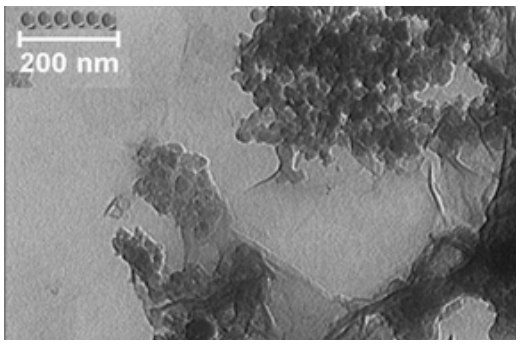

Fig. 2. Transmission Electron Micrograph of the Nanohydroxyapatite synthesized from SCOBY

Table 1. XRF results of materials percentage in kombucha SCOBY powder nanoparticles

Elements	The amount of (%)	Elements	The amount of (%)
Cao	59.38	Fe_2o_3	2.030
K_2o	12.74	Al_2o_3	1.290
So_3	7.92	Cl	0.845
Na_2o	4.73	Tio_2	0.390
Mgo	4.27	Mno	0.343
P_2o_5	2.62	Zno	0.250
Sio_2	2.63	Sro	0.187
		Cuo	0.178

compared to the control group. Although the enzyme activity decreased in the groups treated with nanohydroxyapatite (NHA) compared to the ovariectomy group, this decline was not statistically significant. A comparison was made between the concentration of parathormone hormone in the respective groups and the control group. The findings demonstrated a noteworthy elevation of this hormone in the ovariectomy group; however, in the groups subjected to NHA treatment, an inverse relationship was observed, whereby a decrease in hormone levels ensued in proportion to concentration increments, ultimately approaching levels akin to the control group. The observed decrease did not demonstrate statistical significance across any of the treatment groups in relation to the control group.

The experimental groups exhibited alterations in serum calcium and phosphorus levels relative to the control group; however, these alterations did not achieve statistical significance.

The results displayed in Table 2 indicated that the bone tissue of ovariectomized rats exhibited a significant decrease in calcium concentration in comparison to the control group. However, when comparing the calcium levels between the groups receiving NHA treatment and the ovariectomy group, a noticeable increase in calcium level was found, particularly in the rats exposed to the highest concentration of NHA. The bone phosphorus levels displayed a notable decline across all ovariectomized groups in comparison to the control group. However, upon comparing the phosphorus levels among the ovariectomized rat group with the groups subjected to NHA treatment, it was found that the rats treated with the highest concentration of NHA exhibited a substantial increase in phosphorus concentration. The phosphorus and calcium levels in the liver of the experimental groups exhibited little variation compared to the control group and among themselves, however, significant differences were not observed.

Histopathological results

The photographs A3 and A4 depict a segment of liver tissue of the treated with the highest concentration of NHA captured under magnifications of 40× and 100×, correspondingly.

Table 2. The results of analysis of serum and tissue factors in experimental groups

Groups Parameter	Control group	Ovariectomy group	Ovariectomy+ NHA (25 mg/kg) group	Ovariectomy+ NHA (50 mg/kg) group	Ovariectomy+ NHA (50 mg/kg) group
ALP (U/L)	@@138.67± 57.23	237.67± 30.77**	196.17± 44.31	171.33± 41.40	@154.00± 41.34
PTH (ng/ml)	@@@12.76± 3.50	31.72± 3.69***	27.97± 3.09***	@25.80± 2.49***	@@23.73± 3.49***
Serum Calcium (mg/dl)	11.53± 1.82	13.43± 1.34	13.50± 0.95	13.18± 1.53	12.75± 2.45
Serum Phosphorus (mg/dl)	5.33±1.42	6.77±1.97	5.92±1.25	6.23±1.52	6.40±1.49
Liver Calcium (mg/g tissue)	0.25±0.03	0.19±0.02	0.21±0.02	0.20±0.01	0.22±0.03
Liver Phosphorus (mg/dl)	2.48±0.42	2.14±0.08	2.27±0.30	2.12±0.29	2.31±0.55
Bone Calcium (mg/g tissue)	@@@0.40±0.03	0.23±0.04***	0.24±0.03***	0.24±0.02***	@0.29±0.03***
Bone Phosphorus (mg/g tissue)	@@@9.22±1.22	6.33±0.55***	7.69±0.40*	7.25±0.78**	@@8.27±0.89

The data are expressed as the mean SD. The stars indicate a comparison of the level of factors in the experimental groups relative to the control and the (@) indicate a comparison of the levels of this factors in the ovariectomy group compared to the other groups. The significance level is considered as (* P≤0.05), (**P≤0.01), (***P≤0.001).

There is an absence of any observable tissue destruction, hyperemia, inflammation, or intercellular deposition of elements within the examined sample. Similar to the control group Fig. 3 (A1, A2), lobules, hepatocytes and sinusoids are completely normal. Fig. 3 (A7 and A8) illustrate distinctive sections of the renal tissue of the treated with NHA under two varying degrees of magnification. The renal tubes, Bowman's capsule, and glomerular network exhibited no signs of pathology or dysfunction when compared to the control group Fig. 3 (A5, A6). There are no indications of disorders, such as inflammation, vascular hyperemia, and cell destruction, being present. The accompanying illustrations, denoted as Fig. 3 (A11 and A12), respectively, depict segments of the pulmonary organ in treated groups. the pulmonary alveoli and associated blood vessels indicated uniform and normal morphology, similar to the control group Fig. 3 (A9, A10). The alveoli and tracheas do not exhibit any buildup of elements. No abnormality was observed in the composition of cell chains and spleen tissue pulps in the treatment group Fig. 3 (A15, A16). The tissue appearance in this group was similar to the control group Fig. 3 (A13, A14). The photographs shown were of the rats in the experimental group that received the highest amount of NHA treatment and control group excluding any duplicate images.

The bone tissue results

The Fig. 4 (B1, B4 and B7) are part of bone epiphysis in the control, ovariectomy, and treatment group with the highest concentration of NHA respectively. B1 illustrates a homogeneous and regular texture in a segment of bone tissue from the control group, implying normality. In Fig. 4 (B4) figure, tissue destrucion and irregularity in the ovariectomy group epiphysis is evident. The tissue structure of treatment group Fig. 4 (B7) is almost to the control group. Fig. 4 (B2, B5) and Fig. 4 (B8) show parts of epiphyseal cancellous bone in the control, ovariectomy, and treatment groups respectively at ×100 magnification. In figure Fig. 4 (B2) trabeculae with normal thickness can be seen around the adipose tissue. Destruction of trabeculae and irregularity of adipose tissue cavities are evident in Fig. 4 (B5). A relaitve similarity is observed between the cancellous bone tissue of the NHA treatment group (B8) and the control group. Fig. 4 (B3) shows a part of the bone diaphysis with osteocytes in lacuna in the control. In ovariectomized group Fig. 4 (B6), the majority of the lacunae exhibit an absence of cells. In the image denoted as Fig. 4 (B9), the number of osteocytes in lacuna was higher than in the ovariectomized group. The omission of

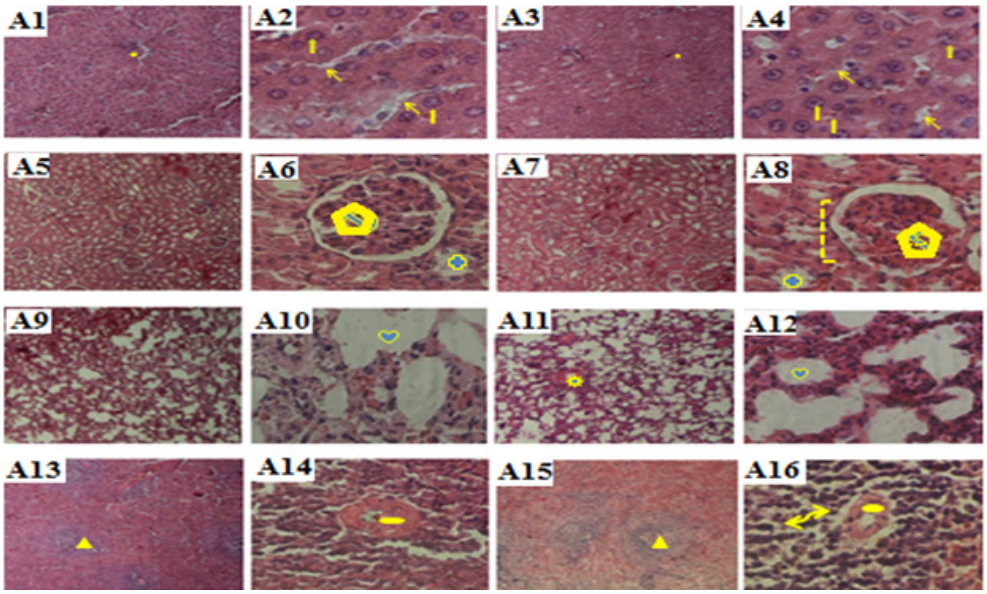

Fig. 3. Histological images of liver (A1 & A2), kidney (A5 & A6), Lung (A9 & A10) and spleen (A13 & A14) in the control group and the imagesof liver (A3 & A4), kidney (A7 & A8), Lung (A11 & A12) and spleen (A15 & A16) in the treated group with NHA (100 mg/kg) at ×40 and ×100 magnifaction. Lobule center (circle), hepatocytes (thin arrow), sinusoid liver (thick arrow), renal tubes (cross), Bowman's capsule (bracket) and glomerular network (pentagon), pulmonary alveoli (heart) and bronchioles (star), white pulp (triangle) and central arteriole (oval), cell chains (double arrow)

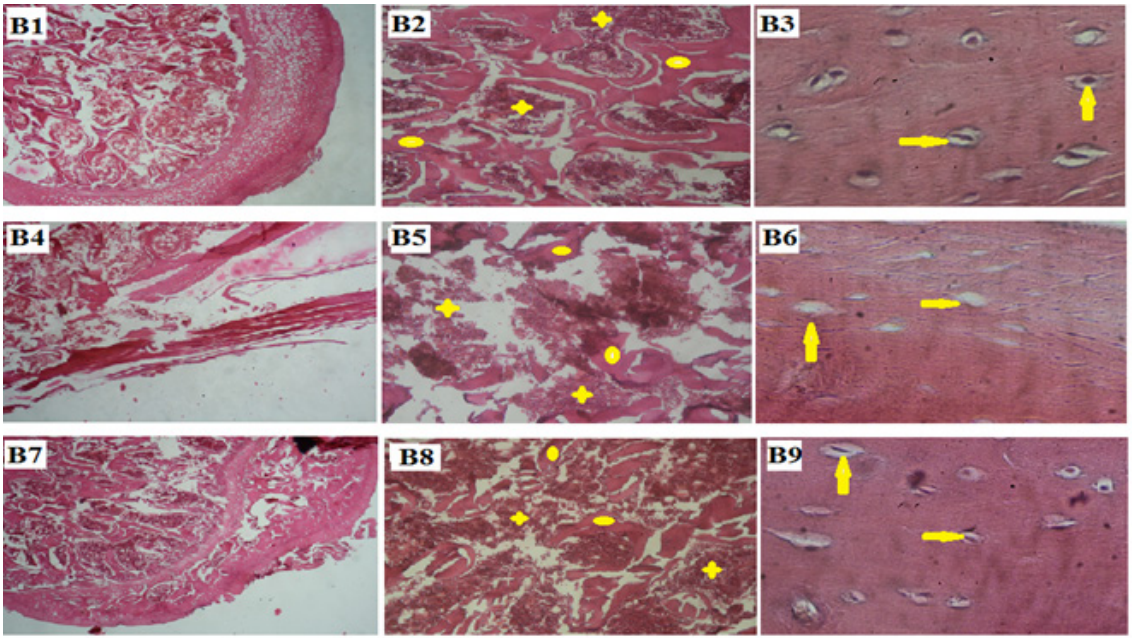

Fig. 4. Histological images of epiphysis of bone (B1, B4, B7) in the control, ovariectomy, and treatment group with the highest concentration of NHA respectively. at ×40 magnifaction. Trabeculae and adipose tissue (B2, B5, B8) at ×100 magnifaction in the control, ovariectomy, and treatment group with the highest concentration of NHA respectively. The bone diaphysis with osteocytes in lacuna (B3, B6, B9) at ×400 magnifaction in the control, ovariectomy, and treatment group with the highest concentration of NHA respectively. Trabeculae(oval), the adipose tissue (star), The osteocytes in lacuna (arrow)

images pertaining to two concentrations, namely 25 mg/kg and 50 mg/kg NHA, was decided upon due to their resemblance to the images from the ovariectomy group.

DISCUSSION

The efficiency of bone density can be increased by combining synthetic hydroxyapatites with organic compounds, as proven by a team of researchers. These materials exhibited superior qualities such as biocompatibility, non-toxicity, and diminutive particle size compared to conventional supplements like calcium carbonate [5, 19, 20]. This study aimed to generate nanohydroxyapatite measuring 30 nm in diameter via the utilization of SCOBY. The XRF results confirmed the presence of more than 50% calcium in SCOBY (Table 1). The confirmation of the synthesis and evaluation of particle size were conducted using XRD and TEM. Prior to the commencement of the principal examination, an assessment was performed on a sample group consisting of 20 rats in order to ascertain the absence of toxicity in this particular nanoparticle. The evaluation involved three distinct concentrations of 25, 50, and 100 mg/kg. Notably, thorough examination of the liver, spleen, kidney, and lung tissues of these animals revealed an absence of any discernible pathological anomalies or abnormalities. The liver, being the largest organ in the body, was examined for its levels of phosphorus and calcium in relation to the control group and treatment groups. An analysis of the liver tissue revealed no evidence of accumulation of these elements, as depicted in Table 2. In accordance with the outcomes of the present study, Remya et al. subjected a total of 140 laboratory rats to a 12-month treatment regimen involving the administration of chemically synthesized nanohydroxyapatite. During the experimental period, there were no indications of abnormalities in hematological biochemical parameters or synogenic toxicity [21]. In a separate investigation, animals were administered hydroxyapatite microcrystals combined with alginate in elevated concentrations of up to 5000 mg/kg, both acutely and subchronically via gastrointestinal delivery. Upon completion of the treatment period, no detectable disruption was noted in the blood parameters and biochemical factors of the tested animals [22]. The present study investigated the impact of the NHA on ovariectomy-induced osteoporosis in rats, following an initial

confirmation of the nanoparticle's nontoxic nature. Initially, a comparison was conducted on the serum ALP activity within the groups under experimentation. The findings indicate a statistically substantial elevation in the enzymatic activity within the ovariectomy group, whereas a decline in ALP activity was observed among other groups compared to the ovariectomy group. This reduction was significant in the treatment group with the highest NHA concentration.

Consistent with the above findings, Hou et al. observed an increase in ALP activity following the induction of osteoporosis in animal with dexamethasone. In this study, the researchers witnessed a discernible reduction in the activity level of ALP subsequent to the administration of Leralic Acid in the rats under investigation [23]. In a separate investigation, the ovariectomy of guinea pigs resulted in a discernible augmentation in alkaline phosphatase activity after a span of two weeks. After treating guinea pigs with Panax ginseng for a duration of ten days, the enzyme's activity returned to normal level [24].

Bone ALP is one of the isoforms of alkaline phosphatase that is released from osteoblasts into the bloodstream, thus playing a crucial role in facilitating bone repair. Hence, it is suitable marker for evaluating bone metabolism [25]. Hence, it seems that the increase ALP activity subsequent to ovariectomy serves as an indication in order to uphold bone structure and expedite the process of bone repair, whereas the decline observed following NHA treatment signifies the impediment of osteoporosis progression.

In addition to evaluating ALP, the level of PTH in the blood was also examined and compared. The findings of this study demonstrated a notable elevation in the hormone levels among ovariectomized rats, while a decrease was observed in the treatment groups compared to the ovariectomy group. An increase in PTH concentration occurs due to the decrease in sex steroid levels after menopause or ovariectomy, suggesting a decline in serum calcium levels [26]. In the present study, serum calcium levels in ovariectomized rats indicated a slight decline. Subsequent administration of NHA resulted in an observed elevation in the concentration of this particular element; however, it did not reach statistical significance. There were no statistically significant changes in the serum phosphorus levels of the experimental groups in comparison to the control group. Elbhasawy et al. observed a decline in the serum concentration of calcium and a concomitant rise in PTH levels in rats subjected to ovariectomy. Following the administration of plant compounds to the aforementioned animals, researchers observed a contrary outcome to their preliminary findings. This prompted the proposition that phytoestrogens present in plants might induce heightened calcium reuptake from the intestinal tract, subsequently contributing to a decline in parathyroid hormone levels [26]. Berlin et al. demonstrated that an augmentation in calcium intake among rats is associated with a subsequent reduction of serum parathyroid hormone (PTH) levels [27]. The study conducted by Bhattarai found no statistically significant variation in the serum concentrations of phosphorus and calcium between menopausal and non-menopausal women [28].

In both the United States and South Korea, two separate studies have shown that phosphorus intake does not contribute to the development of osteoporosis [29, 30]. The discrepancy observed in these outcomes may stem from the multifaceted role of parathyroid hormone. The main effect of this hormone is the lowering of sex steroid levels, while simultaneously enhancing the intake of calcium from the intestine and its reuptake through the kidneys. Nevertheless, the enduring and consequential impact of this hormone pertains to the assimilation of phosphorus and calcium within the skeletal structure to facilitate the process of bone restructuring and repair [31]. The present study demonstrated a noteworthy elevation in calcium and phosphorus levels within the bone of ovariectomized rats following treatment with NHA, particularly at the greatest concentration tested, thereby substantiating this assertion. The current findings suggest that parathyroid hormone exhibited a potential enhancing effect on the intestinal absorption of NHA and subsequent transportation to bone tissue. In a research study, aliquots of 50-100 µL/ml of two distinct hydroxyapatite samples, one chemically synthesized and the other bioengineered, were administered through injections to a group comprising newly hatched chickens. The investigation revealed a noteworthy augmentation in bone mineral density, explicitly notable in the subset subjected to the bioengineered hydroxyapatite treatment [32]. Castelo-Branco et al. have documented that the administration

of hydroxyapatite supplements in tablet form yields considerably superior outcomes compared to calcium carbonate in terms of curtailing the rate of bone mineral density (BMD) decline [3]. Butera et al. developed a toothpaste formulation incorporating hydroxyapatite and subsequently administered it to a group comprising 40 individuals demonstrating white lesions. The researchers' observation of dental remineralization in the individuals discussed herein led them to propose that hydroxyapatite interacts with oral saliva to release Po+4 and Ca+2 ions, thereby ameliorating this particular dental lesion [33].

Lee and Cho conducted a scholarly investigation which revealed an increase in bone mineral density after the intake of calcium and phosphorus [34]. In a separate investigation, an elevation in bone density was recorded in ovariectomized rats subsequent to a three-month course of treatment involving the application of risedronate-synthesized nano hydroxyapatite [35].

Additionally, the experimental groups' bone tissue underwent histopathological evaluation as part of the ongoing research. The findings of the study demonstrated notable enhancements in improvement of tissue disorders following treatment with NHA, particularly at the highest concentration. These positive effects include a reduction in the diameter of fissures and grooves in the bone diaphysis, a decrease in cell death, and an increase in the thickness of the epiphysis bone trabeculae. In accordance with the aforementioned findings, in a scholarly study conducted by the principal investigator and his research team, the effects of rosedronate hydroxyapatite nanoparticles were investigated on ovariectomized rats. Following a one-month treatment period with three varying concentrations of 250, 350, and 500 µg/kg, the researchers observed a notable augmentation in both the trabecular thickness in the diaphysis of the femur and leg, as well as an elevation in trabeculae density [35]. The histopathological findings observed in the current investigation exhibited a correspondence with the outcomes derived from the assessment of other measured variables within the experimental samples.

CONCLUSION

Based on the findings of this study, it can be assumed that the hydroxyapatite synthesized with SCOBY, due to its small size, probebly passed through the small intestine and was absorbed into the bone. In addition, the secondary impact of PTH in this group of rats may have been effective in reducing the rate of bone destruction.

Many supplementary tests are needed to confirm these results.

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CONFLICTS OF INTEREST

The authors report no conflict of interest.

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