Original Article

Investigating The Effect Of Kajwa Herbal Product On Possible Effects On Liver And Osteoporosis Caused By Glucocorticoid In Rats

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Abstract

Osteoporosis is a disease with decreased bone density and loss of bone microstructure, which in turn increases the risk of various fractures. This disease is one of the most important causes of disability in the elderly, especially women. One of the important complications of osteoporosis is pathological fracture, which in some cases can be fatal. Nowadays, various prevention and treatment methods, including pharmacological and non-pharmacological methods (such as alendronate and parathyroid hormone) are available against this disease. Given the chemical nature of the drug compounds and the side effects of current treatments, it seems appropriate to find a herbal alternative. This study was designed and performed to determine the prophylactic effect of Kajwa plant product on glucocorticoid-induced osteoporosis in rats.

Methods: In this study, 35 male Wistar rats weighing in the range of 250-200 g were used in the groups including control, Normal saline 0.9% (negative control) + 0.1 mg / kg dexamethasone by subcutaneous injection, Kajwa herbal composition with a dose of 500 mg / kg + 0.1 mg / kg dexamethasone as a subcutaneous injection, Kajwa herbal composition with a dose of 1000mg / kg + 0.1 mg / kg dexamethasone as a subcutaneous injection, Alendronate (1 mg / kg / day) + 0.1 mg / kg dexamethasone by subcutaneous injection for 2 months.

Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) per liter (U / L) and lipid profile including measurements of serum levels of total cholesterol, HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein) and triglycerides (mg / dL) were measured according to the instructions in each kit. Data were analyzed by SPSS software (19) using descriptive statistical tests (frequency percentage and mean and standard deviation) and ANOVA test in case of abnormal data distribution from its parametric bread equivalent (Kruskal Wallis).

Findings: Comparison of the mean levels of liver enzymes in the studied rats showed that there was no significant difference between the different groups (P> 0.05). The results of serum cholesterol, triglyceride and HDL levels did not show a significant difference between different groups of rats. The results of this study also showed that there was a significant difference in serum creatinine levels between the different groups, so that the group receiving dexamethasone was significantly higher than the other groups (P <0.05). Co-administration of kajwa with dexamethasone improved dose-dependent histology of bone tissue.

Conclusion: Kajwa can have beneficial effects in improving dexamethasone-induced osteoporosis. Also, doses of 500 and 1000 mg / kg of this compound do not have a detrimental effect on the biochemical factors under study.

Keywords: Kajwa, Histology, Oxidative Stress, Osteoporosis, Dexamethasone

INTRODUCTION

Statement of the problem:

Bone is a vital and dynamic tissue that is regenerated to replace old and damaged bones with stronger new bones. This takes places with the coordinated activities of osteoclasts and osteoblasts. Many bone related diseases are caused due to disorders in bone regeneration. Osteoporosis is one of these diseases (1).

Osteoporosis is a systemic skeletal disorder. It happens because of a decrease in bone mass and the deterioration of the microscopic structure of the bone tissue. These lead to an increase in bone fractures and its further sensitivity (2).

10.3% of the American adults aged over 50 suffer from osteoporosis (3). Nowadays, osteoporosis is known as an important health and treatment problem and it has been called “the silent disease of the century” due to its asymptomatic nature. The complications of this disease can cause lots of irreparable financial and physical losses to the patients and society. This is why the World Health Organization has declared the decade from 2000 to 2010 as the decade of bone and joint diseases, including osteoporosis (4, 5). The rate of osteoporosis affliction is increasing and according to the studies conducted about it in different parts of the world, about 75 million people in Europe, Japan and the United States are suffering from this disease (6). In Iran, according to the statistics of the Rheumatology Research Center of Tehran University of Medical Sciences, 6 million Iranians are suffering from osteoporosis (7).

Menopause and glucocorticoids are among the main causes of osteoporosis (8). Glucocorticoids are widely used as a treatment for many inflammatory disorders, autoimmune diseases (such as rheumatoid arthritis, systemic lupus erythematosus), pulmonary disorders (bronchial asthma and chronic obstructive pulmonary disease), tumors, and organ transplantation (9, 10). On the other hand, these glucocorticoid drugs have serious side effects in the long run. These side effects are glaucoma (gradual blindness), diabetes, osteoporosis, obesity, muscle disorders, and cardiovascular diseases (11, 12). Fractures caused by osteoporosis are one of the most common side effects of the long-term use of glucocorticoids. This includes 30 to 50 percent of the patients who take glucocorticoids (13). Oxidative stress is probably one of the important factors in the pathogenesis of the bone damage which is related to age (14). The increase of oxidative stress is accelerated by the increase of the duration of glucocorticoid treatment (15). Glucocorticoids increase inflammatory cytokines such as TNF alpha (16). Affecting the signaling pathways in bone regeneration directly by gamma receptors, Glucocorticoids increase the rate of bone damage and the risk of fractures (17, 18).

The Kajwa herbal drug, which was produced by the company of Kajwa therapeutic products in Iran, in 2018, consists of various herbal compounds. Plants have antioxidant properties (19, 20). Kajwa as an herbal medicine has some herbal compounds such as Nigella Sativa (21, 22), Shilajit (23) and chamomile honey (24, 25) whose antioxidant properties have been investigated and proven in previous studies. The presence of a very strong antioxidant in this compound controls the free radicals and stops the reaction of free radical chains.

The importance of the beneficial or harmful effect of any drug and treatment, including herbal medicines, on liver enzymes and lipid profile is very high. Considering the evidence in traditional medicine and the studies conducted on the antioxidant property of the compounds of Kajwa and the lack of studies done about the effect of similar compounds on liver activity and lipid profile, we designed and performed this study to determine the preventive effect of this herbal drug on osteoporosis caused by glucocorticoid in rats.

LITERATURE REVIEW

Kajwa herbal drug is a completely new drug and there are no studies about it on the available scientific databases as far as we know. So in this part, we present the very similar studies to ours.

Ding et al. did a study in 2018 titled “Methylbenzokism as a therapeutic agent for the osteoporosis caused by glucocorticoid in rats". They induced an osteoporosis model in the rats by injecting 0.1 mg/kg dexamethasone for sixty days. Then the rats were divided into the following five groups randomly. Each group had five rats.

1. Control 2. No treatment 3. Treatment with 2 mg/kg methylbenzokism 4. Treatment with 5 mg/kg methylbenzokism 5. Treatment with 10 mg/kg methylbenzokism

After sixty days, the rats of the three treatment groups received 2, 5 and 10 mg/kg methylbenzokism daily for fifteen days. During the same time, the rats of the “Control” and “No treatment” groups received the same amount of normal saline instead of methylbenzokism. The results showed that methylbenzokism controls the osteoblast proliferation and increases osteoprotein activity. It also decreases the receptor activator for nuclear factor kappa-B. Dexamethasone decreases protein activity of the Wnt signaling in the osteoblast. Although dexamethasone exposes osteoblasts to receive methylbenzokism, it controls the Wnt signaling protein in osteoblasts inversely. Pathological studies on the femur show that treatment with methylbenzokism improves the effects of dexamethasone. In the rats suffering from osteoporosis, methylbenzokism increases osteocalcin significantly. The amount of this increase depends on the amount of methylbenzokism. It also decreases the type one collagen fragments compared to the No treatment group. These effects have been significantly observed ($P < 0.05$) in 5 and 10 mg/kg treatment groups compared to the 2 mg/kg treatment group. As a result, methylbenzokism prevents dexamethasone-induced osteoporosis in rats (26).

Azadeh Javadi et al. conducted a study to investigate the histopathological and histomorphometric effect of Sargassum tennerimum on the bone tissue of rats suffering from osteoporosis. This experimental study was conducted on 80 NMRI laboratory rats. The rats were divided into Control, Sham, Positive control, Intervention and Negative control groups after inducing osteoporosis to them. The therapeutic dose of the Positive control group was 125 mg/kg of calcium and 0.025 microgram/week/mice of vitamin D, and it was 10 mg/100 g of body weight per day of Sargassum tennerimum extract in the Intervention group, which was fed to each group for 28 days. At the end, histopathology sections were studied. The Negative control group showed the most destruction

in bone blades compared to other groups ($P < 0.01$). The Intervention group had less destruction than the Negative control group and the number of osteoblasts seen in the periosteum was significantly increased ($P < 0.01$) compared to the negative control group (27).
Jiang et al. conducted a study in 2015 titled "Effects of electromagnetic pulse field on bone structure and lipid metabolism in rats suffering from osteoporosis induced by glucocorticoid through the standard Wnt protein signaling pathway". They divided forty rats into four groups. Each group contained 10 rats. A dose of 2.5 mg/kg dexamethasone was injected to thirty of these rats twice a week for twelve weeks to induce osteoporosis to them. Then they were divided into three groups: a group receiving 56.25 mg/kg calcium supplement daily; a group receiving electromagnetic pulse field with a frequency of 50 Hz and an intensity of 4 MT daily for 40 minutes, and the osteoporosis group. They were investigated for twelve weeks. The results showed that a significant increase in bone mineral density (BMD) and a significant decrease in the amount of serum lipids in the electromagnetic-pulse-field receiving group compared to the osteoporosis group. Also bone trabeculae were thicker in electromagnetic-pulse-field receiving group. In the electromagnetic-pulse-field receiving group, the activity of the Wnt10b, LRP5, catenin ،βOPG and Runx2 increased significantly ($P < 0.05$) and the activity of Axin2 ،PPAR-γ ،C/EBPα ،FABP4 and Dkk-1 decreased significantly ($P < 0.05$).
The results of this study show that the use of electromagnetic pulse field can prevent the decrease of bone density in rats suffering from osteoporosis and improve the metabolism of lipids (28).
Abdel Fattah et al. did a study titled "the Effects of bisphosphonate on alveolar bone of rats suffering from glucocorticoid-induced osteoporosis". They divided thirty rats into three groups of ten rats: 1. Control 2. Osteoporosis group who received a dose of 0.6 mg/kg dexamethasone twice a week for twelve weeks. 3. Treatment group with bisphosphonate who received a dose of 0.6 mg/kg dexamethasone twice a week for twelve weeks + the gavage of 1 mg/kg risedronate daily. The results showed that the alveolar bone has been worsened in the osteoporosis group compared to the control group. This bone had a significant structural and ultra-structural improvement in the treatment group with bisphosphonate (risedronate) compared to the osteoporosis group. In addition, EDX (Energy dispersive X-ray) analysis showed a significant decrease ($P < 0.05$) in the amount of calcium in the osteoporosis group compared to the control group. Also, in the treatment group with bisphosphonate, the amount of calcium was returned to normal and comparable values to the control group (29).
Kamaruzzaman et al. conducted a study in 2019 titled "Improving glucocorticoid-induced osteoporosis by using the antioxidant property of Clovelot honey". They divided thirty five rats whose adrenal glands had been removed into five groups. The groups were: Healthy, Control, two groups of receiving 200 and 400 mg/kg Clovelot honey as a daily gavage and a group of positive control receiving a daily 2% calcium in water solution. All the groups, except the healthy group, received daily intramuscular injections of 120 µg/kg dexamethasone to induce osteoporosis. This was done for two months. The results showed that there was a significant increase in malondialdehyde and a significant decrease in superoxide dismutase in the groups under treatment. But no significant change was observed in the in the activity of catalase and glutathione peroxidase. Bone and trabecular volumes decreased significantly while the trabecular part of the femur increased. This is in harmony with the reduction of osteoblast level after two months of glucocorticoid administration. Clovelot honey caused the superoxide dismutase to return to the normal level and reduced the amount of malonindialdehyde significantly. Moreover, this honey increased bone and trabecular volumes and decreased the trabecular part volume significantly which was confirmed by increasing the level of osteoblasts and decreasing the level of osteoclasts (30).

Objectives & Hypotheses:

General objectives:
Determining the protective effect of Kajwa herbal drug on osteoporosis caused by glucocorticoid in rats

Specific objectives:
1- Comparison of the average serum level of liver parameters of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes among five studied groups on the 60th day of the study.
2- Comparison of the average serum level of different lipid profile parameters (total cholesterol, HDL, LDL and triglyceride) among five studied groups on the 60th day of the study.
3- Comparison of the average calcium serum level among five studied groups on the 60th day of the study.

Applied objectives
The purpose of this study is to determine the effectiveness of an herbal product called Kajwa to obtain a method and a drug to prevent the damage caused by osteoporosis. If it is obtained, we can decrease the costs and harms of the usual treatment drugs.

Hypotheses / Questions

1- Comparison of the average serum level of liver parameters of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes among five studied groups on the 60th day of the study shows a difference.
2- Comparison of the average serum level of different lipid profile parameters (total cholesterol, HDL, LDL and triglyceride) among five studied groups on the 60th day of the study shows a difference.
3- Comparison of the average calcium serum level among five studied groups on the 60th day of the study shows a difference.

RESEARCH METHODS

In this study, 35 Wistar male rats weighing between 200-250 gr were used. These rats were kept in stable physical conditions in the Animal house. The temperature was 25 ± 2 degrees Celsius. The light condition was also a normal 12-hour light and dark cycle. These rats had free access to food (Khorasan Sprout Company) and water. They were divided into groups seventy-two hours before when the study started. Then they were put in the new cages in order to adapt themselves to the new condition. Each group contained seven rats.
Group 1: 0.9% Normal saline recipient (Control)
Group 2: 0.9% Normal saline recipient (negative control) + 0.1 mg/kg dexamethasone (26) (Aburaihan Pharmaceutical Company, Tehran, Iran) by hypodermic injection for two months.
Group 3: 500 mg/kg Kajwa herbal plant recipient by gavage + 0.1 mg/kg dexamethasone by hypodermic injection for two months.
Group 4: 1000 mg/kg Kajwa herbal plant recipient by gavage + 0.1 mg/kg dexamethasone by hypodermic injection for two months.
Group 5: 1 mg/ kg/ day Alendronate recipient (manufactured by Aveh Sina Pharmaceutical Company, Iran) by gavage + 0.1 mg/kg dexamethasone by hypodermic injection for two months.
After the treatment period, the rats were kept fasting for 12 hours. Then, they were anesthetized by ketamine (80mg/kg) and xylazine (100mg/kg) and blood was drawn from them through cardiac puncture. After that, their serum was separated by centrifugation at 2500 rpm for 10 minutes. We kept them at minus 20 degrees Celsius in order to measure liver enzymes and biochemical factors.
Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) per liter (U/L) and Lipid profile including the measurement of serum levels of total cholesterol, HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein) and triglycerides (mg/dl) were all measured according to the instructions in each kit.
Doing a pathological evaluation on the collected blood samples, we dissected the femur bone samples of the rats. Then we separated a sample of their liver to examine histologically and kept it in 10% formalin solution for 72 hours. We prepared the tissues as follows:

1. Dehydration: To perform this operation, the tissue is soaked in alcohol (70, 80, 96 and 100) for 1 hour.
2. Clearing and alcohol extraction: To perform this operation, xylene was used for three hours (1.5 hours in each container).
3. Paraffin saturation: To perform this operation, paraffin with a melting point of 45 to 60 degrees Celsius was used for 3 hours.
4. Molding: To keep the sample easily and simplify the cutting, the samples were molded in the paraffin mass by the Leukhardt mold.

The tissue was cut by a microtome machine. The provided slices were 3 to 5 microns thick. They were stained with hematoxylin and eosin (H & E) and evaluated.

Study design & Method:

Type of study:

Experimental

Data collection techniques:

Laboratory data obtained from blood serum test results as well as histological results of bone tissue

Sampling method & Sample size calculation:

The non-probability sampling method was randomly done and the sample size was based on similar studies that had been done before.

Data analysis:

To achieve the purpose of the study, SPSS Statistics (version 19) was used to analyze the collected data. If the data had a normal distribution and were parametric, ANOVA was used, otherwise they were analyzed Kruskal-Wallis test at a significant level of $P<0.05$.

Variables Table

number	Variable name	Type of variable	Variable role (dependent-independent-distorting...)	Scientific definition	Method and unit of measurement
1	Kajwa herbal drug	Qualitative-Nominal	independent	plant extract consumption in a certain amount	mg/kg
2	ALT enzyme	Quantitative-continuous	dependent	ALT enzyme level in the serum of the tested sample, which is expressed by a number	U/L
3	AST enzyme	Qualitative-Nominal	dependent	AST enzyme level in the serum of the tested sample, which is expressed by a number	U/L
4	serum total cholesterol	Qualitative-Nominal	dependent	total cholesterol level in the serum of the tested sample, which is expressed by a number	mg/dl
5	HDL	Qualitative-Nominal	dependent	HDL level in the serum of the tested sample, which is expressed by a number	mg/dl
6	LDL	Qualitative-Nominal	dependent	LDL level in the serum of the tested sample, which is expressed by a number	mg/dl
7	triglyceride	Qualitative-Nominal	dependent	Triglyceride level in the serum of the tested sample, which is expressed by a number	mg/dl
8	calcium	Qualitative-Nominal	dependent	Calcium level in the serum of the tested sample, which is expressed by a number	U/L

9	Rat group	Quantitative-continuous	dependent	Laboratory animals used in the experiment where a specific intervention is performed in each group.	Types of studied groups

Ethical considerations:

Ethical considerations were considered in all stages of the current research. These considerations are related to the animals. They are:

1) The correct selection of animal species
2) Using the minimum number of animals wherever possible
3) providing suitable living conditions for animal
4) Training researchers about the life and physiological conditions of animals (nutrition, health, disease, discomfort and pain and other physiological and pathological changes of animals)
5) Not using sick animals in the experiment
6) Considering ethical protocols in blood sampling
7) Providing conditions for an easy death for the animals at the end of the study

Design limitations, possible systematic errors and ways to deal with them:

Although there were some losses in the tested rats, we did not come across a serious problem because we had anticipated it before starting the study.

RESULTS

The results of serum levels of liver enzymes among different groups of studied rats are shown in Table 1 and Chart 1.

Table 1: Comparison of the average serum level of liver enzymes in the studied rats

	AST	**ALT**
control	156.18 ± 45.76	96.58 ± 6.54
Dexa (0.1mg/kg)	111.41 ± 19.32	92.05 ± 12.51
Kajwa (500 mg/kg)	132.31 ± 35.44	80.55 ± 8.63
Kajwa (1000 mg/kg)	135.83 ± 45.33	84.08 ± 10.19
Aln (1 mg/kg)	134.78 ± 23.54	85.84 ± 16.30
The result of the statistical test	F=1.16 P=0.35	F=2 P=0.12

The data in the table represent the mean ± standard deviation.

According to the above table and comparing the level of liver enzymes in the studied rats, there is no significant difference among the different studied groups (P>0.05).

The results of serum levels of cholesterol, triglyceride and HDL among different groups of studied rats are shown in Table 2.

Table 2: Comparison of the average serum level of the serum lipid profile of the studied rats.

	triglyceride	**Total cholesterol**	**HDL**	**LDL**
control	62.5 ± 17.38	53.67 ± 7.81	32.33 ± 5.27	21.33 ± 3.01
Dexa (0.1mg/kg)	67.5 ± 27.71	47 ± 6.81	26.17 ± 4.02	20.83 ± 3.65
Kajwa (500 mg/kg)	56.5 ± 18.07	57.5 ± 7.81	32.67 ± 4.84	24.83 ± 3.25
Kajwa (1000 mg/kg)	47.67 ± 12.32	57.5 ± 15.52	34.17 ± 9.98	23.33 ± 6.21
Aln (1 mg/kg)	67.2 ± 22.75	49.6 ± 4.27	29.4 ± 2.6	20.2 ± 4.08
The result of the statistical test	F=0.99 P=0.43	F=1.46 P=0.24	Chi-Square=7.76 P=0.1	Chi-Square=4.28 P=0.36

The data in the table represent the mean ± standard deviation.
According to table 2 and comparing the average level of lipid profile in the studied rats, there is no significant difference among the different studied groups (P>0.05).
The results of serum levels of uric acid, creatinine and calcium among different groups of studied rats are shown in Table 3.

Table 3: Comparison of the average level of the serum uric acid, creatinine and calcium of the studied rats.

	uric acid	**creatinine**	**calcium**
control	1.43 ± 0.63	0.41 ± 0.06[a]	8.33 ± 0.66
Dexa (0.1mg/kg)	2.42 ± 2	0.57 ± 0.12[b]	8.56 ± 1.06
Kajwa (500 mg/kg)	1.38 ± 0.66	0.41 ± 0.03[a]	8.41 ± 0.34
Kajwa (1000 mg/kg)	1.4 ± 1.17	0.34 ± 0.05[a]	8.11 ± 0.95
Aln (1 mg/kg)	1.48 ± 0.78	0.4 ± 0.08[a]	8.18 ± 1.12
The result of the statistical test	Chi-Square=1.81 P=0.76	Chi-Square=12.2 P=0.01	F=0.25 P=0.9

The data in the table represent the mean ± standard deviation. The letters “a”and “b” indicate the significant difference in the results of the follow-up test between the groups.
According to the above table and comparing the average level of serum uric acid and calcium in the studied rats, there is no significant difference among the different studied groups (P>0.05).
The results showed that there is a significant difference in the serum level of creatinine among the different studied groups. So that, this amount was significantly higher in the group receiving dexamethasone compared to other studied groups (P<0.05).

Histopathological results

Control group

Histological studies showed that the bone tissue is normal in this group. Bone trabeculae were firm, intact and thick and they were covered with a continuous layer of osteoblasts. Also narrow bone marrow spaces were observed with a large amount of hematopoietic tuberculosis and a small amount of fat cells (Figure 1).

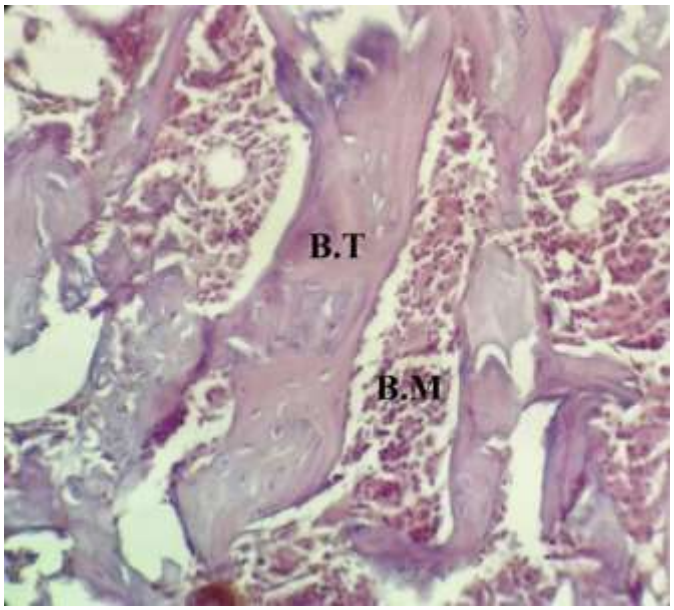

Fig 1: Intact and thick bony trabeculae (B.T) with regular surface lined by continuous layer of osteoblasts associated with narrow bone marrow spaces (B.M). H&E, X 400

Dexamethasone group

The histological evaluation of this group showed that trabeculae were thin, irregular and even broken with a discontinuous surface of osteoblasts. They were surrounded by large bone marrow spaces with fat infiltration. Bone erosion by multinucleated osteoclasts was also visible in this group (Figure 2).

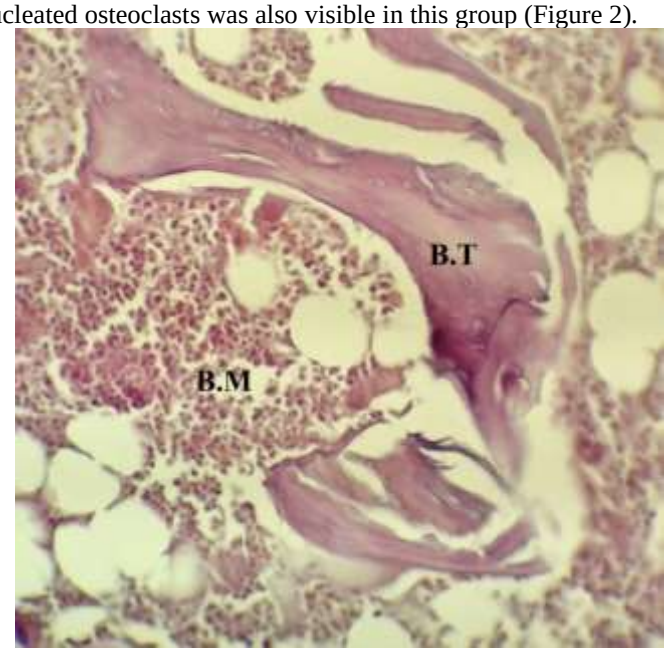

Fig 2: Thin, irregular and even fractured bony trabeculae (B.T) with discontinuous of surface osteoblasts which were surrounded by wide bone marrow spaces (B.M) associated with fatty infiltration. H&E, X 400

500 mg/kg Kajwa group

Histological studies showed that the bone returned normal in thickness and structure. The bone surface was relatively continuous and was surrounded by narrow bone marrow spaces (Figure 3).

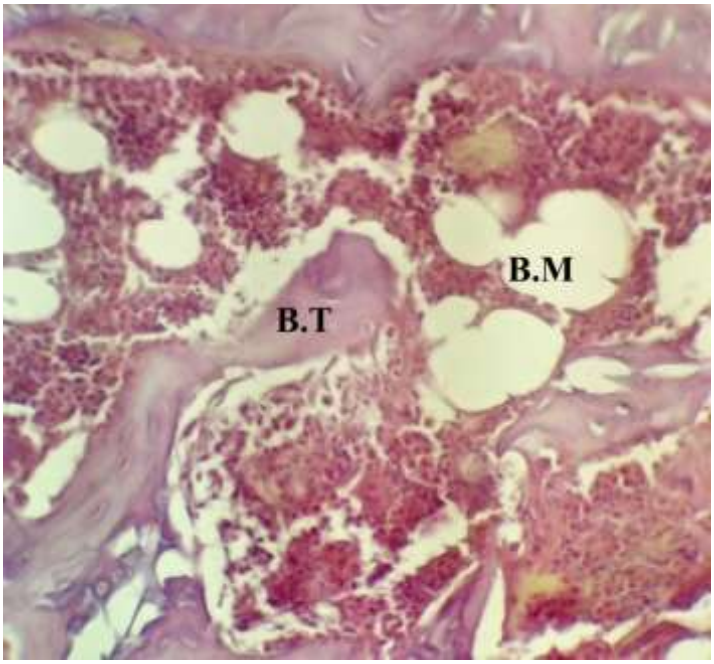

Fig 3: Bone trabeculae (B.T) returned slight normal in thickness and structure and relatively continuous bone surface with narrow bone marrow spaces (B.M). H&E, X 400

1000 mg/kg Kajwa group

Histological studies in this group showed that the bone returned slightly normal in thickness and structure. The bone surface was also relatively continuous and was surrounded by narrow bone marrow spaces (Figure 4).

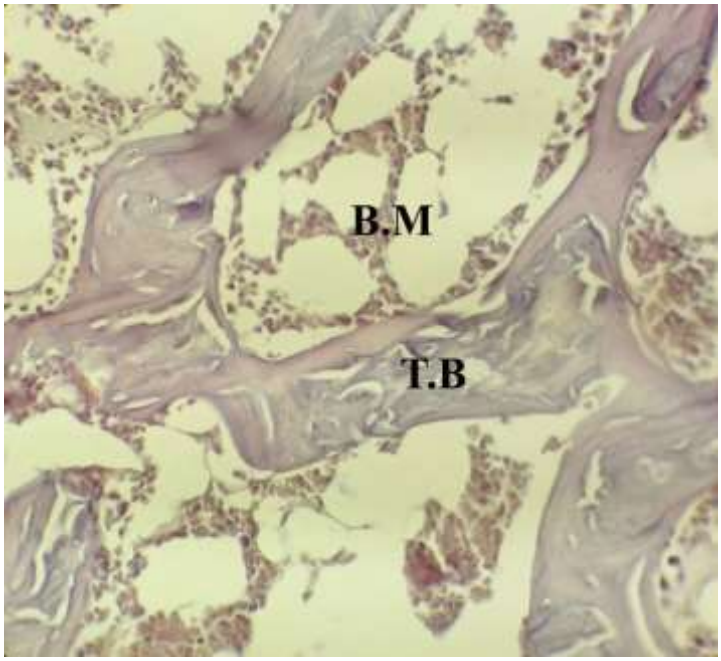

Fig 4: Bone trabeculae (B.T) returned slight normal in thickness and structure and relatively continuous bone surface with narrow bone marrow spaces (B.M). H&E, X 400

Alendronate group

Histological studies of this group showed that the bone returned normal in thickness and structure. The bone surface was relatively continuous and was surrounded by narrow bone marrow spaces (Figure 5).

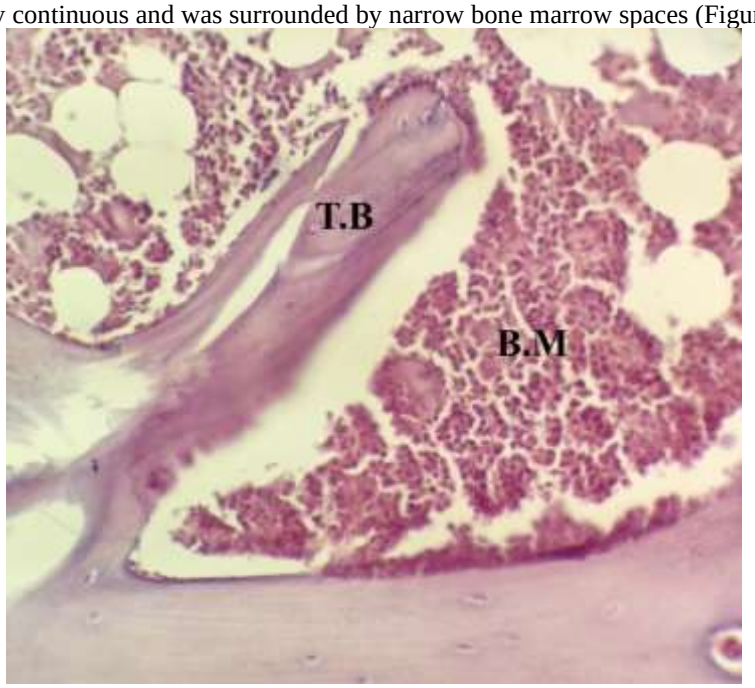

Fig 5: Bone trabeculae (B.T) returned slight normal in thickness and structure and relatively continuous bone surface with narrow bone marrow spaces (B.M). H&E, X 400

DISCUSSION AND CONCLUSION

In this study, we investigated the protective effect of Kajwa herbal drug on osteoporosis caused by dexamethasone. We compared the effects of this herbal drug with the effects of alendronate, which is a common drug for osteoporosis. The mentioned Kajwa contains Nigella Sativa, wax, honey and royal jelly.

Dexamethasone is a long-acting corticosteroid with strong anti-inflammatory effects. It is well-known for creating an animal model of osteoporosis (26, 30-32). Glucocorticoids cause osteoporosis by several mechanisms such as: reducing the gastrointestinal absorption of calcium, controlling osteoblasts, increasing the activity of osteoclasts

by changing RANKL production. In this study, in line with other studies, daily injection of dexamethasone could change the rats' femur bones by creating thin and irregular trabeculae with discontinuous surface osteoblasts and fat infiltration. Although dexamethasone increased the level of serum creatinine significantly, it caused no significant changes in other investigated factors (33-35).

Dose-dependent co-administration of Kajwa and dexamethasone improved the histology of the bone tissue. As the treatment effect of the combination of these four drugs had not been investigated before, we searched through the studies that had investigated the effect of each one separately and compared their results to ours. The observed effects are in line with previous studies conducted on Nigella Sativa. Ezirganli et al. reported that daily administration of 10 mg/kg Nigella Sativa for six weeks improves bone regeneration, decreases osteoclasts and increases osteoblasts in rats suffering from osteoporosis caused by Ovariectomy (36). Also, the administration of 5 and 10 mg/kg thymoquinone- the main active substance of Nigella Sativa- reduces inflammatory factors, controls the activity of osteoclasts and produces osteoprotective effects in rats. (37). But unlike animal studies, Nigella Sativa did not show a beneficial effect in a short-term human study conducted on postmenopausal women by Valizadeh et al. in (38). However this difference may be because of the short duration of the study, the complex nature of the disease in humans, the small number of patients and the inappropriate form of the drug (oil).

We think that some of the observed effects in our study is related to the available Shilajit in Kajwa. Various studies have reported the beneficial effects of this compound (Shilajit) on osteoporosis. In an animal study, rats suffering from alcohol-induced osteoporosis were significantly improved by administering this compound. These effects also caused some changes in antioxidant enzymes (39, 40). Also, its administration in a clinical trial of patients suffering from tibia fractures improved the recovery time significantly (41).

Moreover, some of the observed effects caused by Kajwa are related to honey and royal jelly. Heideka et al reported the anti-osteoporosis effects of honey and royal jelly in ovariectomized rats. They reported an increase in bone calcium and bone density but no change in osteoclast production (42). Also, Narita et al reported an increase in osteoblasts caused by oral administration of royal jelly to healthy rats (43). Lots of studies have been conducted about the effects of honey on bones. Yudaniayanti et al. also reported an increase in bone density in ovariectomized rats caused by honey administration (44). Kamaruzzaman et al. believe that honey is useful for osteoporosis because of quercetin, kaempferol, gallic acid and ascorbic acid. These materials have anti-inflammatory and antioxidant properties. They can also prevent osteoclast activity and increase osteoblast activity which both lead to an increase in bone density. 45).

In addition to investigating tissue effects, liver enzymes including alanine aminotransferase and aspartate aminotransferase, lipid profile, uric acid and calcium levels, and kidney biochemical profile including serum urea and creatinine were also examined. It was found out that taking to 1000 mg/kg of this compound does not cause any significant changes in the blood factors except for the dose-dependent effect on creatinine. Kajwa reduced the increase of creatinine caused by dexamethasone significantly. In our study, dexamethasone only caused a significant increase in creatinine, but in Hasano et al.'s study, apart from this increase, it increased fasting blood sugar, ALT, AST and decreased albumin (46). Kajwa probably applies its beneficial effects on creatinine through Nigella Sativa and honey. Dala et al. conducted a study that showed adding high doses of Nigella Sativa to rats' diet for five weeks causes a significant decrease in creatinine compared to its low doses in the control group (47).

CONCLUSION

The findings of this study showed that Kajwa herbal drug, which contains Nigella Sativa, honey, Shilajit and royal jelly, can improve the bone tissue and strengthen the bone density in osteoporosis caused by dexamethasone but it does not have any effect on the studied biochemical factors. Also, 500 and 1000 mg/kg doses of this compound do not have a harmful effect on the investigated biochemical factors. It is worth to mention that identifying the exact mechanism of this compound requires more studies and investigation about bone tissue signaling pathways.

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