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ASSESSMENT OF NIGELLA SATIVA OIL IN DEXAMETHASONE INDUCED OSTEOPOROSIS IN MANDIBLE OF WISTER ALBINO RATS: EXPERIMENTAL STUDY

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ABSTRACT

Objective: to investigate the effect of Nigella sativa oil on Dexa induced osteoporosis in Wister albino rats' mandible.

Material and Methods: Thirty Wister albino rats were divided into five groups. C group served as control group. D group; rats received injections with Dexa at a dose of 5 mg/kg I.P. for two weeks. ND group; the rats received injections with Dexa 5 mg/kg I.P for two weeks and intraoral Nigella sativa oil (5 ml/kg). N group; the rats received 5 ml/kg intraoral Nigella sativa oil for two weeks. NDD group; rats receiving I.P injections of Dexa 5 mg/kg for 4 weeks, the rats were given intraoral doses of Nigella sativa oil 5 ml/kg every day in the last 2 weeks. Blood levels of parathyroid hormone and calcium were assessed. Rats were euthanized by cervical dislocation then dissection each rats' mandible, Hematoxylin and eosin (H&E) stain and special stains with Alizarin red and Von-Kossa.

Results: N group showed the strongest positive reaction for both Von-kossa and Alizarin red stains. One-way ANOVA test, detected the significant differences between five groups Meanwhile, the highest mean value was found in the N group. On the other hand, the lowest mean value was found in the D group. By one-way ANOVA test, the highest value of calcium was existed in N group (10.44±0.56) and while the lowest value was found in D group

Conclusion: The results of this study demonstrated that NS oil was beneficial for preventing and treating mandibular osteoporosis induced by Dexa.

KEY WORDS: Nigella sativa (NS); Dexamethasone (Dexa), Von-kossa and Alizarin red

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INTRODUCTION

Dexamethasone (Dexa) is a non-selective glucocorticoid (GC) drug that manages inflammatory, allergic, and immunological diseases that is done by activating nuclear GC receptors [1][2]. On the other hand, long-term high doses of Dexa had many side effects such as insulin resistance hyperglycemia, and hyperlipidemia as a result of great changes in metabolism associated with Dexa administration [3].

Prolonged use of Dexa may also lead to secondary osteoporosis and sometimes to osteonecrosis which leads to bone fracture [4][5]. However, Den et. al., 2011 state that more than 30% of patients who take Dexa for a long time suffer from different degrees of bone fracture [6]. as our knowledge goes, osteoblasts play an important role in bone regulation and formation [7]. However, GCs had a target to inhibition of osteoblast proliferation and change in its activity and that is known as GC-induced osteoporosis [8 - 10].

Nigella sativa (NS), is one of the herbal seeds that contain high increments of minerals such as Ca, P, Fe and Zn, Mg, Ma, Mn, and copper in lesser amounts [11]. NS is considered one of the medical plants that can aid in osteoporosis treatment [12]. However, NS had a great pharmacological effect as an antidiabetic, anticancer, analgesic, anti-inflammatory, and antioxidant ability [13,14,15]. So, when using NS extract with a bone graft to fill a bone defect it can improve bone healing and bone regeneration [16]. Thymoquinone (TQ), is the extract of NS that has most of the therapeutic effect of these seeds. TQ is also found to be the most active chemical ingredient of the essential oil. However, NS acts as a food flavoring agent which had a very low level of toxicity [17].

The aim of this study is to investigate the effect of NS oil on Dexa-induced osteoporosis using Special stains; Alizarin red and Von-kossa to differentiate between mineralized and unmineralized bone in different groups and blood analysis to assess the level of Calcium and parathyroid PTH in various groups.

MATERIAL AND METHODS

The experimental processes were performed under the protocols of the Ethics Committee of Faculty of Oral and Dental Medicine, Delta University (FaDMRC-2023-00101)

Study design: thirty male Wister albino rats, three months old, weighing between 190 and 220 grams, were obtained from the Experimental Research Center at Mansoura University Egypt (MERC). At MERC, they were placed in separate cages. They were fed a regular food, given water, and kept in a 12-hour light/12-hour dark cycle. Five groups of six rats each were randomly selected.

I) Group I (G1, C group): the rats were kept in the same housing and fed as test rats served as a negative control.

II) Group II (G2, D group): the rats received daily injections of 5 mg/kg I.P. Dexa for two weeks (AMRIYA for Pharmaceutical Industries Alexandria - Egypt).

III) Group III (G3, ND group): the rats received intraoral Nigella sativa oil (5 ml/kg) for two weeks parallel with injections of Dexa 5 mg/kg I.P. (to investigate the role of NS oil in preventing the osteoporosis induced by Dexa)

IV) Group IV (G4, N group); the rats received 5 ml/kg intraoral Nigella sativa oil for two weeks,

V) Group V (G5, NDD group): The rats received I. P injections of Dexa 5 mg/kg I.P for 4 weeks and they were given intraoral doses of Nigella sativa oil (5 ml/kg) for the final two weeks (to investigate the role of NS oil in the treatment of the osteoporosis induced by Dexa). After two weeks from starting the study; blood samples were obtained in order to measure the levels of parathyroid hormone (PTH) and calcium (Ca), except for the fifth group, after four weeks. All of the rats were euthanized via cervical dislocation under anesthesia. All the rats'

mandibles were dissected, and the soft tissues were removed. Dissected bone was Fixed in a solution of 10% formalin; Ethylene diamine tetra-acetic acid solution (EDTA) (Microdec edta-based, diapath S.p. A, Martineengo BG, Italy) was used for decalcification. The specimens were processed into paraffin blocks at the Pathology Department, Faculty of Medicine, Mansoura University. They were prepared for histological examination using hematoxylin and eosin (H&E) stain (Sigma Aldrich) for routine examination. Special stains such as Alizarin red and Von-kossa stains (Sigma-Aldrich Co.) were used to identify mineralized from unmineralized bone.

Staining techniques for Alizarin Red and Von-Kossa; deparaffinized tissue slides were first rinsed in distilled water and stained with alizarin red solution for 30 seconds to 5 minutes, dehydrated in acetone, 20 dips and in Acetone-Xylene (1:1) solution, 20 dips. Slides were cleared in xylene and mounted. Positive reaction appeared as red-orange or brown discoloration[18]. For **Von-Kossa** deparaffinized tissue slides were first rinsed in distilled water then slides were Incubated in silver nitrate solution (5%) for 60 minutes, rinsed with distilled water, incubated in Sodium Thiosulfate Solution (5%) for 5 minutes. Slides were rinsed (2 minutes in running tap water) dehydrated, cleared and mounted. Positive reaction areas appeared as a dark brown or black coloration[19].

Statistical analysis and data interpretation

Collected data regarding surface area reaction of mineralized bone and staining intensity (strong-weak) were accomplished using image analysis as well as blood analysis were fed to the computer and analyzed using GraphPad Prism 8 (GraphPad Software). Data were analyzed for normality by using test of normality (Shapiro-Wilk test). Laboratory results data showed normal (parametric) distribution. Data were presented as mean, and standard deviation (SD) values. The One-way ANOVA followed by post hoc Tukey's multiple comparison test were used to compare between the groups. The significance of the obtained results was judged at the (0.05) level.

RESULTS

Alizarin red stain

Positive surface area (mineralized bone) was measured as well as staining intensity, we found that, D group had the weakest reaction in comparison with other groups. On the other hand, the N group had the strongest reaction in all groups. In one-way ANOVA detect the significant differences between five groups (F ratio = 22.08 and P value =< 0.0001). Meanwhile, the highest mean value was found in the N group (72.90 ±4.94). On the other hand, the lowest mean value was found in the D group (46.86 ±8.27). The mean value of the control group is (65.03 ±6.33), while the mean value of the ND group is (58.90 ±6.86), and finally, the NDD group's mean value is (61.31< ±5.03). Tukey's multiple comparisons post hoc test revealed significant differences between the D group and the other four groups ($P < 0.05$). Also, there is a significant difference detected between the N group ND group, and NDD group ($P < 0.05$). Meanwhile, there are insignificant differences between the control group and the other three groups N, ND, and NDD (Table 1, 2 and 3) (Fig. 1) (Diagram I).

Von Kossa special stain

N group revealed the strongest positive reaction to the stain while the D group showed the weakest reaction to the stain. All other groups had a positive reaction to the Von Kossa stain. One-way ANOVA test was used in this stain by calculating the surface area percentage (mineralized bone), the highest value is for the N group (60.62±5.19) and the lowest value is for the D group (37.92±6.73). The other group's values for C, ND, and NDD groups were (57.86±7.31), (51.31±7.55) and (50.60±8.23) respectively. However, there are significant differences between each other's (F ratio

= 15.41 and P value =< 0.0001). Tukey's multiple comparisons post hoc test revealed significant differences between the D group and the other four groups ($P < 0.05$). Also, there is a significant difference detected between the N group ND group, and NDD group ($P < 0.05$). Meanwhile, there are no significant differences between the control group and N, ND, and NDD groups (Table 4,5) (Fig. 2) and 6) (Diagram II).

Blood test for calcium level and parathyroid hormone

By one-way ANOVA test, the highest value in calcium and parathyroid hormone analysis was for N group (10.44±0.56) and (4.02±0.15) while the lowest value was for D group (7.51±0.41) and (2.25±0.30) respectively in row. The other group's values for C, ND, and NDD groups were (9.01±0.89) and (3.88±0.17), (8.90±0.50) and (3.20±0.38), and (8.81±0.58) and (3.40±0.20) respectively. However, there are significant differences between each other's (F ratio = 17.41 for Ca, 44.59 for PTH and P value =< 0.0001). Tukey's multiple comparisons post hoc test for calcium level revealed significant differences between D group N group and C group ($P < 0.05$) (Table. 7).

TABLE (1) Surface area percentage of Alizarin red staining expression represented by mean and standard deviation.

Alizarin red	Control	Dexamethasone (D)	Nigella sativa (N)	N+D	N+D+D	F ratio	P value
Surface areas percentage							
Mean	65.03	46.86	72.90	58.90	61.31	22.08	<0.0001*
SD	±6.33	±8.27	±4.94	±6.86	±5.03		
Mean gray value							
Mean	155.64	189.44	149.22	161.49	159.94	47.06	<0.0001*
SD	±7.79	±7.19	±7.00	±7.57	±5.90		

Data is presented as Mean and ± Standard Deviation (SD) ***Used test: One-Way ANOVA***

****: Significant at P < 0.05***

TABLE (2) Post-hoc Tukey's multiple comparisons test statistical results for the different groups Alizarin red staining.

Compared groups	P value
Control vs. Dexamethasone	<0.0001*
Control vs. Nigella sativa	0.0626
Control vs. N+D	0.2211
Control vs. N+D+D	0.6934
Dexamethasone vs. Nigella sativa	<0.0001*
Dexamethasone vs. N+D	0.0011*
Dexamethasone vs. N+D+D	<0.0001*
Nigella sativa vs. N+D	0.0001*
Nigella sativa vs. N+D+D	0.0018*
N+D vs. N+D+D	0.9158

****: Significant at P < 0.05***

TABLE (3) Post-hoc Tukey's multiple comparisons test statistical results for the different groups Alizarin red staining Mean gray value.

Compared groups	P value
Control vs. Dexamethasone	<0.0001*
Control vs. Nigella sativa	0.2749
Control vs. N+D	0.3652
Control vs. N+D+D	0.6623
Dexamethasone vs. Nigella sativa	<0.0001*
Dexamethasone vs. N+D	<0.0001*
Dexamethasone vs. N+D+D	<0.0001*
Nigella sativa vs. N+D	0.0032*
Nigella sativa vs. N+D+D	0.0129*
N+D vs. N+D+D	0.988

****: Significant at P < 0.05***

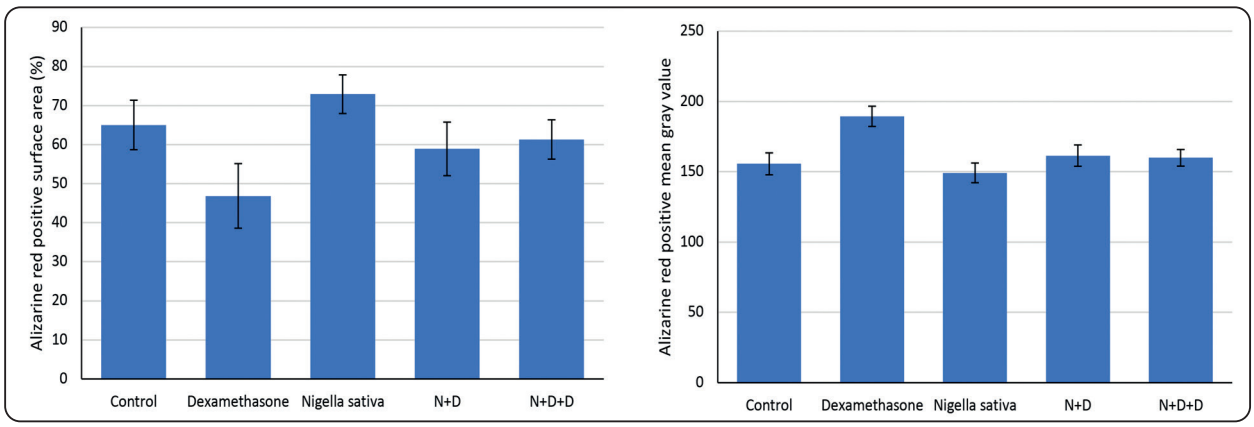

Diagram I: Alizarine red positive surface area % and mean value.

TABLE (4) Surface area percentage of Von Kossa staining expression represented by mean and standard deviation.

Von Kossa	Control	Dexamethasone (D)	Nigella sativa (N)	N+D	N+D+D	F ratio	P value
Surface areas percentage							
Mean	57.86	37.92	60.62	51.31	50.60	15.41	<0.0001*
SD	±7.31	±6.73	±5.19	±7.55	±8.23		
Mean gray value							
Mean	132.82	174.99	128.00	150.00	156.63	19.3	<0.0001*
SD	±19.94	±12.66	±11.65	±9.86	±11.80		

Data is presented as Mean and ± Standard Deviation (SD) ***Used test: One-Way ANOVA***

****: Significant at P < 0.05***

TABLE (5) Post-hoc Tukey's multiple comparisons test statistical results for the different groups Von Kossa staining surface area percentage.

Compared groups	P value
Control vs. Dexamethasone	<0.0001*
Control vs. Nigella sativa	0.9052
Control vs. N+D	0.2512
Control vs. N+D+D	0.1654
Dexamethasone vs. Nigella sativa	<0.0001*
Dexamethasone vs. N+D	0.001*
Dexamethasone vs. N+D+D	0.002*
Nigella sativa vs. N+D	0.0391*
Nigella sativa vs. N+D+D	0.022*
N+D vs. N+D+D	0.9994

****: Significant at P < 0.05***

TABLE (6) Post-hoc Tukey's multiple comparisons test statistical results for the different groups Von Kossa staining Mean gray value.

Compared groups	P value
Control vs. Dexamethasone	<0.0001*
Control vs. Nigella sativa	0.9321
Control vs. N+D	0.0531
Control vs. N+D+D	0.0028*
Dexamethasone vs. Nigella sativa	<0.0001*
Dexamethasone vs. N+D	0.0016*
Dexamethasone vs. N+D+D	0.0331*
Nigella sativa vs. N+D	0.0066*
Nigella sativa vs. N+D+D	0.0002*
N+D vs. N+D+D	0.8122

****: Significant at P < 0.05***

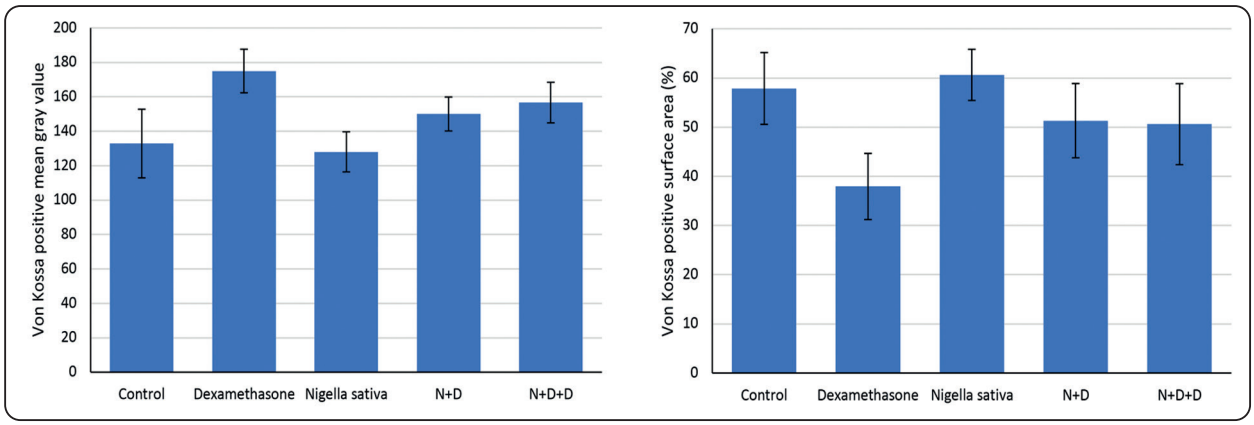

Diagram II: Von Kossa positive surface area % and mean value.

TABLE (7) Ca and PTH mean and slandered deviation:

	Control	Dexamethasone	Nigella sativa	N+D	N+D+D	F ratio	P value
Ca (mg/dl)	9.01±0.89	7.51±0.4^{1}a	10.44±0.5^{6a}b	8.90±0.5^{0b}c	8.81±0.5^{8b}c	17.41	<0.0001
PTH (pg/mL)	3.88±0.17	2.25±0.3^{0}a	4.02±0.1^{5}b	3.20±0.3^{8ab}c	3.40±0.2^{0ab}c	44.59	<0.0001

Values expressed as mean ± SD.

Used test: One way ANOVA followed by post hoc Tukey's multiple comparison test.

a: Significance Vs. Control, b: Significance Vs. Dexamethasone, c: Significance Vs. Nigella sativa at p < 0.05.

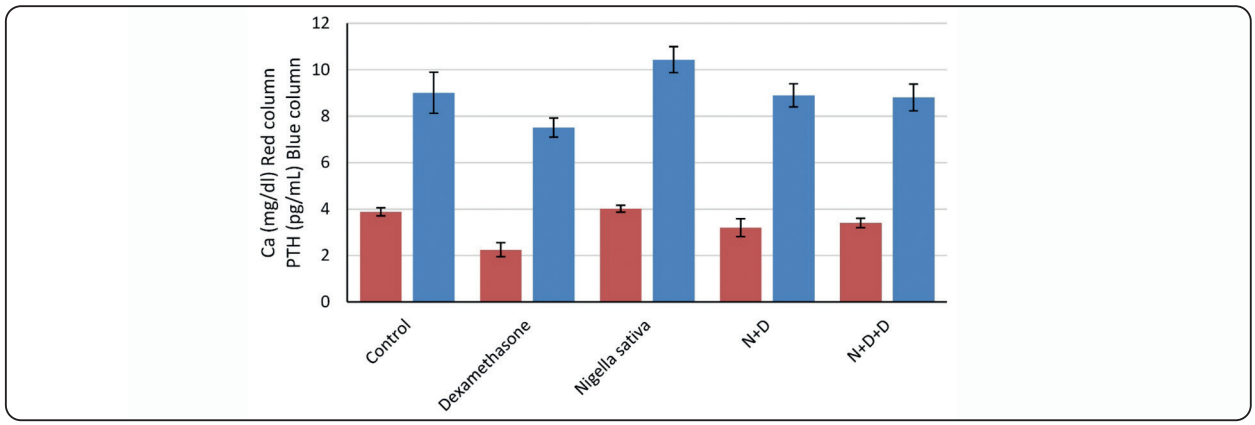

Diagram III: Ca and PTH level

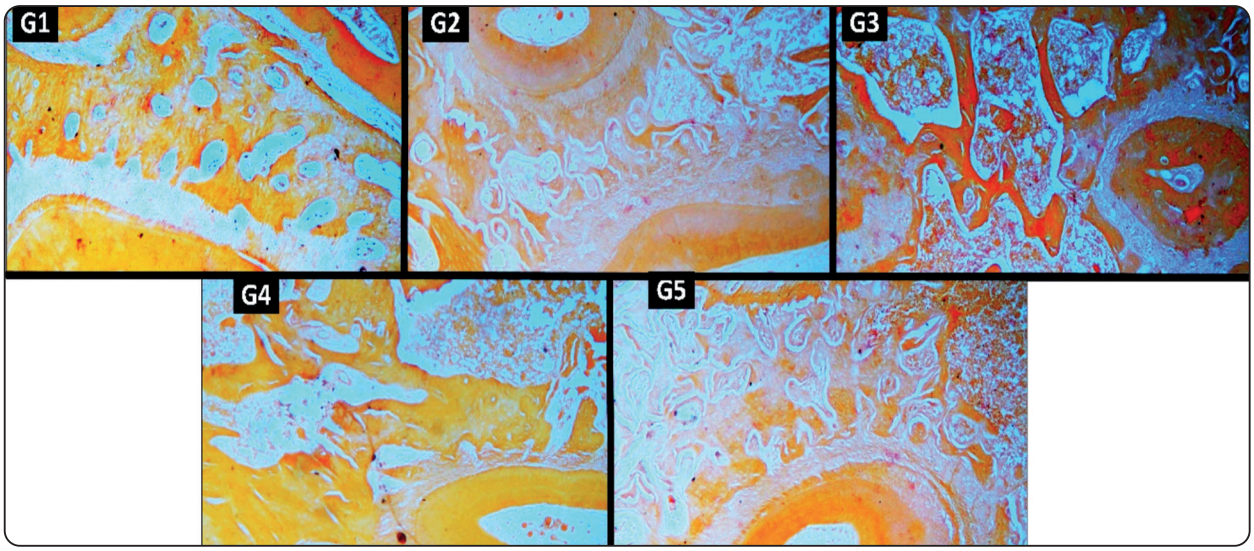

Fig. (1) Decalcified section of rat mandible showing the intensity of Alizarine red special stain, the strongest reaction for the N group (G3) and the weakest reaction for the D group (G2), (G1) control, ND (G4) and NDD (GS) groups showed strong reaction to alizarin red, Alizarin red special stain 1.00x.

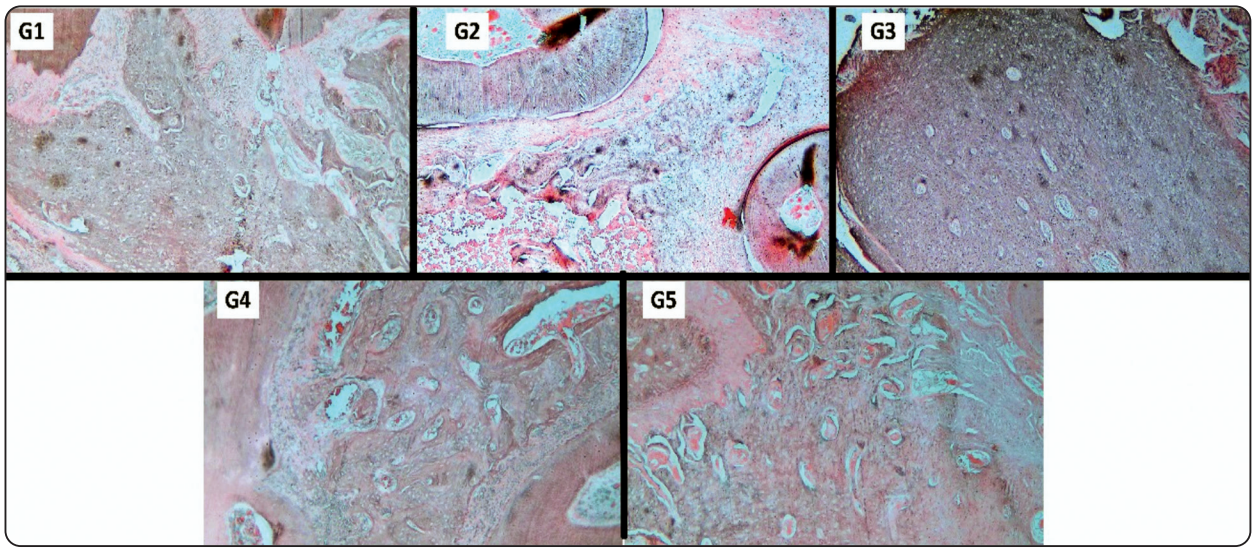

Fig. (2) Decalcified section of rat"s mandible showing strong positive reaction of N group (G3) to Von Kossa special stain, positive reaction to control (G1), ND (G4) and NDD (G2) groups, and weak reaction of D group (G2), Von Kossa special stain 100x.

DISCUSSION

For thousands of years, plants had been used for health and medicinal purposes. It is difficult to determine the exact date of the first plant medicine use, since carbon dating from ancient Babylon (Iraq) revealed that plants were cultivated 60 000 years ago [20]. Plants have been used much more in the last decade for grain conservation as well as for the prevention and treatment of animal health issues. The development of organic livestock production systems, drug resistance, high input costs, and concerns about toxic residues in food are causing problems. Other reasons are also found in the side effects of traditional drugs [21]. The World Health Organization (WHO) has recently identified traditional medicine (including herbal drugs) as a collection of therapeutic practices that have existed

for a long time [20], thus, the main objective of this research is to evaluate whether or not NS works to protect and reverse osteoporosis caused by Dexa.

In this study, we have used Dexa to induce osteoporosis in rats by reducing bone formation and increase of bone resorption[22] This was in agreement with several studies, Liping et al stated that, Dexa had the ability to alter the microarchitecture of bone with long-term use causing osteoporosis by decreasing the bone formation due to the inhibition of osteoblasts proliferation and promoting of their apoptosis [23]. Elsyade et al observed in dexa treated rat model numerous bone cavities, increased the number of apparent enlarged empty osteocyte lacunae. In addition, an uneven endosteum was found, along with an erosion cavity harboring several multinucleated osteoclasts. Also, there were lightly stained collagen fibers by MT staining indicating a decrease in the bone density [24]. Also, Sivagurunathan et al demonstrated that the administration of corticosteroids causes bone fragility and osteoporosis due to lowering protein in the bone matrix and increased osteoclast formation [25]. Liu et. al. (2014), who used Dexa, methotrexate, and celecoxib, they found that Dexa had severe side effects on the metabolism of bone [26]. Another study demonstrated the side effect of Dexa on bone metabolism found that rats suffered from a reduction in total alkaline phosphatase enzyme (~600 IU/L) in addition to hyperglycemia and reduction in serum lipid [27].

For understanding the effect of NS on osteoporosis we used Alizarin red and Von Kossa special stains. Alizarin red special stain has a strong affinity for calcium ions and can aid in the visualization of bone and the illustration of its mineral composition [28]. However, Von Kossa special stain acts by replacing tissue calcium bound to phosphate with silver ions. So, silver ions were visualized after hydroquinone was reduced to metallic silver [29].

In our study results, we examined the bone of rats' mandible. There is a similarity in the result between Alizarin red and Von Kossa special stain. the D group, revealed the weakest reaction to the stain in comparison to the N group which showed the strongest reaction, this could explain low calcification due to osteoporosis induced by Dexa in D group. The other three groups control, ND, and NDD had strong reactions to both stains in this study. Several studies investigated the influence of NS and reported its positive benefits on osteoporosis and bone repair and agreed with our results, Ezirganli et. al. (2016) as they used NS extract for calvaria bone defect in female rats after ovariectomized. They found histologically that NS extract aids in bone healing in comparison to the control group [16]. Also, seif (2014) used NS for 4 weeks then did ovariectomy and reused NS for another 8 weeks. he found that NS can overcome osteoporosis happen due to ovariectomy [30].

Other studies by, Elsyade et al, demonstrated that NS improved the altered bone structure, and NS group showed well-distinct cement lines indicating bone remodeling and improvement of bone strength [24]. Also, Musa et al., stated that, NS protect against progressive osteoblast apoptosis and can be used therapeutically to reduce anti-inflammatory and antioxidant effects while enhancing the detoxification process [31].

Similar results on NS's impact on OP reported that NS can either have an antioxidant effect by scavenging free radicals and/or by inhibiting lipid peroxidation, which both help to mitigate the bone damage brought on by diabetes [32]. Numerous earlier studies concluded that NS can be used to treat postmenopausal OP, and diabetes-induced OP, and to promote fracture repair [33- 35].

Our results revealed serum calcium level in N group within normal as control group, this was in acceptance with, Elsyade et al who, demonstrated that rats received NS showed more improvement in serum Ca and vitamin D and they clarified that by the following: first, NS enclose valuable amounts of calcium, so, it is considered a natural source of calcium intake for kids, the elderly, pregnant women, and breastfeeding mothers[24].

Another clarification was highlighted by Winkler who reported the biochemical, pharmacological, immunological, and anti-inflammatory effects of NS[36]. According to Al-Mutheffer's study in rabbits, applying NS oil extract as a percutaneous therapy improves bone repair by promoting cell migration and differentiation processes and concluded, NS increases the production of the extracellular matrix and organizes its calcification [37]. Also, TQ decreased osteoclast number and raised osteoblastic activity [38]. However, Valizadeh et al studies were unsuccessful in displaying the valuable influences of short-time intake of NS extract on bone turnover, so they did not advise it for remedial use in OP [39].

Based on the current results we can conclude that NS oil had a good effect on osteoporosis induced by Dexa in Wister rat's mandible and that confirmed by Von-kossa, Alizarin red special stains and blood calcium level. Thus, it is recommended to use NS oil for the protection and treatment of Dexa-induced osteoporosis.

REFERENCES

1. Rafacho A, Ortsäter H, Nadal A, Quesada I. Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. J Endocrinol. 2014;223:R49–62.
2. Wu D-Y, Ou C-Y, Chodankar R, Siegmund KD, Stallcup MR. Distinct, Genome-Wide, Gene-Specific Selectivity Patterns of Four Glucocorticoid Receptor Coregulators. Nucl Recept Signal. 2014;12:nrs.12002.
3. Hopkins RL, Leinung MC. Exogenous Cushing's Syndrome and Glucocorticoid Withdrawal. Endocrinol Metab Clin North Am. 2005;34:371–84.
4. Weinstein RS. Glucocorticoid-Induced Bone Disease. N Engl J Med. 2011;365:62–70.
5. Kerachian MA, Séguin C, Harvey EJ. Glucocorticoids in osteonecrosis of the femoral head: A new understanding of the mechanisms of action. J Steroid Biochem Mol Biol. 2009;114:121–8.
6. den Uyl D, Bultink IEM, Lems WF. Advances in Glucocorticoid-Induced Osteoporosis. Curr Rheumatol Rep. 2011;13:233–40.
7. Yang M, Huang Y, Chen J, Chen Y, Ma J, Shi P. Activation of AMPK participates hydrogen sulfide-induced cyto-protective effect against dexamethasone in osteoblastic MC3T3-E1 cells. Biochem Biophys Res Commun. 2014;454:42–7.
8. La Corte R, Trotta F, Adami S. Glucocorticoid Receptors and Bone. Curr Pharm Des. 2010;16:3586–92.
9. Briot K, Roux C. Glucocorticoid-induced osteoporosis. RMD Open. 2015;1:e000014–e000014.
10. Li X, Hu C, Zhu Y, Sun H, Li Y, Zhang Z. Effects of Aluminum Exposure on Bone Mineral Density, Mineral, and Trace Elements in Rats. Biol Trace Elem Res. 2011;143:378–85.
11. Verna C, Dalstra M, Wikesjö UME, Trombelli L. Healing patterns in calvarial bone defects following guided bone regeneration in rats. J Clin Periodontol. 2002;29:865–70.
12. Osman MT. Nigella Sativa Has Beneficial Effect on Osteoporosis and Bone Healing. Is It Fact or Fiction? A Systematic Review. Asian J Pharm Clin Res. 2017;10:41.
13. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013;3:337–52.
14. Hmza AJA, Osman MT, Adnan A, Omar E. Immunomodulatory effect of Nigella sativa oil in the disease process of type 1 diabetic rats. Res J Pharm Biol Chem Sci. 2013;4:980–8.
15. Hmza AJA, Omar E, Adnan A, Osman MT. Nigella sativa oil has significant repairing ability of damaged pancreatic tissue occurs in induced type 1 diabetes mellitus. Glob J Pharmacol. 2013;7:14–9.
16. Ezirganli S, Kazancioglu HO, Ozdemir H, Inan DS, Tek M. The Effects of Nigella Sativa Seed Extract on Bone Healing in an Experimental Model. J Craniofac Surg. 2016;27:1905–9.
17. Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA. Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats. J Ayub Med Coll Abbottabad. 2008;20:25–7.
18. Abedin E, Lari R, Mahdavi Shahri N, Fereidoni M. Development of a demineralized and decellularized human epiphyseal bone scaffold for tissue engineering: A histological study. Tissue Cell. 2018;55:46–52.

19. Aboulfotoh M, Zedan A, Elmoslemany A. The Effect of Drinking Magnetic Water in Comparison to Water Treated by Microwave Oven on Bone Minerals Changes. Egypt Dent J.

20. Kuralkar P, Kuralkar SV. Role of herbal products in animal production – An updated review. J Ethnopharmacol. 2021;278:114246.

21. ESCOSTEGUY AP. Potential use of medicinal plants in animal production : results in Brazil. Proc 4th ISOFAR Sci Conf 'Building Org Bridg Org World Congr 2014, 13-15 Oct, Istanbul, Turkey (eprint ID 23725. 2014;:13–5.

22. Lukert BP, Raisz LG. Glucocorticoid-Induced Osteoporosis. Rheum Dis Clin North Am. 1994;20:629–50.

23. Liping An, Liqiang Shi, Yujie Ye, Dankai Wu, Guangkai Ren, Xiao Han, Guangyu Xu, Guangxin Yuan, Peige Du. Protective effect of Sika Deer bone polypeptide extract on dexamethasone-induced osteoporosis in rats. Electronic Journal of Biotechnology, 2021; 52: 52-58

24. Rania H. Elsyade, Heba Abd Elrazak Abd Elfatah, Noha Osama Shawky and Eman A. El-Sawaf. The Putative Role of Nigella Sativa Oil Versus Calcium and Vitamin D in Modulating the Glucocorticoid-induced Osteoporosis in Rats: Histological and Immunohistochemical Study. AIN SHAMS MEDICAL JOURNAL.2023; 74, 833-850

25. Sivagurunathan, S.; Muir, M.; Brennan, T.; Seale, J. and Mason, R. Influence of glucocorticoids on human osteoclast generation and activity. J. Bone Miner Res. (2005);20(3):390- 398.

26. Liu Y, Cui Y, Chen Y, Gao X, Su Y, Cui L. Effects of dexamethasone, celecoxib, and methotrexate on the histology and metabolism of bone tissue in healthy Sprague Dawley rats. Clin Interv Aging. 2015;:1245.

27. Malkawi AK, Alzoubi KH, Jacob M, Matic G, Ali A, Al Faraj A, et al. Metabolomics Based Profiling of Dexamethasone Side Effects in Rats. Front Pharmacol. 2018;9.

28. 28. Ding J, Yan R, Wang L, Yang Q, Zhang X, Jing N, et al. Using Alizarin Red Staining to Detect Chemically Induced Bone Loss in Zebrafish Larvae. J Vis Exp. 2021. https://doi.org/10.3791/63251.

29. Zhu S, Ehnert S, Rouß M, Häussling V, Aspera-Werz R, Chen T, et al. From the Clinical Problem to the Basic Research—Co-Culture Models of Osteoblasts and Osteoclasts. Int J Mol Sci. 2018;19:2284.

30. Seif AA. Nigella Sativa reverses osteoporosis in ovariectomized rats. BMC Complement Altern Med. 2014;14:22.

31. Musa, D.; Duisiz, N.; Gumushan, H.; Ulakoglu, G. and Muharrem, B. (2004). Antitumor activity of an ethanol extract of Nigella Sativa seeds. Biologia Bratislava. 59:635- 670

32. Shady AM, Nooh HZ. Effect of black seed (Nigella sativa) on compact bone of streptozotocin induced diabetic rats. Egypt J Histol 2010;33(1):168-77.

33. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed 2013;3(5):337-52.

34. Shuid AN, Mohamed N, Mohamed IN, Othman F, Suhaimi F, Ramli ES, et al. Nigella sativa: A potential antiosteoporotic agent. Evi Based Complement Alternat Med 2012;2012:Article ID: 696230.

35. Ansari R, Gheek N. Effects of Nigella sativa against osteoporosis. Int J Pure Appl Biosci 2013;1(2):6-14. 38. Ali MA, Abu Sayeed M, Shahinur Alam M, Yeasmin Mst S, Khan AM, Muhamad II: Characteristics of oils and nutrient contents of nigella sativa linn. and trigonella foenumgraecum seeds. Bull Chem Soc Thiop 2012,26(1):55–64

36. Winkler, C.; Schroecksnadell, K.; Ledochowski, M.; schennach, H.; Houcher, B. and Fuchs, D. (2008). Invitro effects of Nigella Sativa seeds extracts on stimulated peripheral blood mononuclear cells. Pteridines. 19(4): 101- 106.

37. Al-Mutheffer EA. The percutaneous effect of black seed (Nigellasativa) oil as external topical treatment on bone healing in rabbits.AL-Qadisiya J Vet Med Sci 2014;13(2):146-54.

38. Ozdemir H, Kara MI, Erciyas K, et al: Preventive effects of thymoquinone in a rat periodontitis model: a morphometric and histopathological study. J Periodontal Res 2012, 47(1):74–80

39. Valizadeh N, Zakeri HR, Asnafi GA, Shafiee A, Sarkhail P, Heshmat R, et al. Impact of black seed (Nigella sativa) extract on bone turnover markers in postmenopausal women with osteoporosis. DARU J Pharm Sci 2009;17 Suppl 1:20-5.