The effect of Rutin hydrate on Glucocorticoids induced osteoporosis in mandibular alveolar bone in Albino rats (Radiological, histological and histochemical study)

Nuha Abdul-Fattah Baraka a, Naglaa Fathallah Ahmed b,*, Safaa Ismail Hussein a

a Oral Biology Department, Faculty of Dentistry, Ain Shams University, Egypt
b Oral Radiology Department, Faculty of Dentistry, Ain Shams University, Egypt

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Abstract  Background: Glucocorticoids are used in different conditions such as autoimmune disorders and organ transplantation and their administration is the most common cause of secondary osteoporosis. Rutin is a flavonoid found in many plants. Flavonoids are natural products with various therapeutic and biological effects.

Objective: Is to investigate the effect of Rutin Hydrate as a form of Rutin on glucocorticoid induced osteoporosis in mandibular alveolar bone radiologically, histologically and histochemically.

Methods: Twenty-one adult male Albino rats were randomly divided into three groups. Group I (control), group II (osteoporotic) and group III (Rutin Hydrate treated). In both group II and III rats received 21 mg/kg of methylprednisolone daily for four weeks. Then group III received 50 mg/kg of rutin hydrate in distilled water daily for another four weeks. At the end of the experiment, mandibles were dissected for radiographic assessment, then processed for histological and histochemical examination and statistical analysis.

Results: Radiologically, administration of Rutin Hydrate was able to enhance bone density than osteoporotic group. Histological examination revealed preserved cortical bone thickness that had been statistically proved. Apparently normal sized marrow cavities, some plump osteoblasts and normal osteocytes were seen in group III. Histochemical examination showed statistical increase in the area percentage of newly formed collagen in group III than group II.

* Corresponding author.
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1. Introduction

Osteoporosis is distinguished by decreased bone strength with increased bone fracture possibility. Bone ability to withstand mechanical forces and fractures depends on bone tissue quantity and quality. Decreased bone mass with deteriorated bone tissue result in increased bone fragility causing fractures with minimal traumas, accompanied with mortality and morbidity (Akesson, 2003; Martin and Correa, 2010).

Synthetic glucocorticoids (GCs) are used in autoimmune disorders, after organ transplantation, malignancies, pulmonary and gastrointestinal diseases (Canalis et al., 2007). Adverse skeletal events are the most common GC-related complications. Glucocorticoids-induced osteoporosis (GIO) results from the anti-inflammatory effect of GCs (Nash et al., 2011). The most common cause of secondary osteoporosis and the cause of non-traumatic osteonecrosis is GC intake. In patients with long-term therapy, GCs encourage fractures in 30 to 50% and osteonecrosis in 9 to 40% (Weinstein, 2012). Combining higher dose and continuous use of GCs for different disorders, had the greatest effect on fractures occurrence (Steinbuch et al., 2004).

Peng et al. (2021) documented that besides vitamin D and calcium suplementations, the major therapeutic options approved for GIO include bisphosphonates, parathyroid hormone N-terminal fragment teriparatide and the monoclonal antibody denosumab.

Rutin (3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonoid found in buckwheat, apple and tea (Hosseinzadeh & Nassiri-Ast, 2014). Flavonoids are abundant natural products with multiple therapeutic biological effects (Mahmoud et al., 2019).

Rutin could diminish liver damage after induced liver toxicity in rats through its antioxidant effects (Gelen et al., 2017). Rutin administration revealed a protective effect against induced hepato-renal and testicular disturbances in rats through its antioxidant and hypolipidemic effects (Elsawy et al., 2019).

Rutin hydrate also showed protective effect through its antioxidant and anti-inflammatory actions in induced acute lung injury in rats (Aktas et al., 2017). It also inhibited neuronal depressant activity in central nervous system mediated by caffeineism in mice (Nema and Bairagi, 2017).

Thus the current study aimed to investigate the effect of Rutin Hydrate on induced osteoporosis both radiographically and histologically.

2. Materials and methods

2.1. Animals

Twenty one male adult Albino Wistar rats between 200 and 250 g were housed in separate cages in Animal House of “Medical Research Center” in Ain Shams University through-out experimental duration. Rats were kept under good ventilation and adequate stable diet. This study was conducted after receiving an ethical clearance from Research Ethics Committee of Ain Shams University, Faculty of Dentistry; the study follows guidelines of the committee. Ethical committee approval number is (FDASU-IR022215).

2.2. Chemicals

- Methylprednisolone succinate as powder and liquid (Solu-Medrol™, Pfizer Manufacturing, Belgium NV, Puurs - Belgium).
- Rutin Hydrate as powder (Sigma Chemical Co., St. Louis, USA).

2.3. Experimental Design:

Rats were randomly divided into three groups; seven rats each:

1-GI, control group: where rats did not receive drugs.
2-GII, osteoporosis group: rats received 21 mg/kg of subcutaneous injection of methylprednisolone daily for four weeks (Wang et al., 2018).
3-GIII, Rutin Hydrate treated group: rats were treated at first as group II followed by oral administration of 50 mg/kg of rutin hydrate dissolved in distilled water daily for four weeks (Kamalakkannan and Prince, 2006).

Rats were sacrificed after eight weeks from experiment beginning by over dose of anaesthesia and mandibles were dissected and fixed in 10% buffered formalin for five days. Afterwards, each mandible was divided into two halves.

2.4. Radiological examination

After fixation, right and left halves of mandibles were imaged using x-ray machine (X-RAY DE GOTZEN X-MIND®) at (70 Kvp and 0.1 mAs) and Photostimulable phosphor imaging plate (PSP) then radiographic images were displayed using Digora Software (DIGORA® Optime DXR-50 001) (Mikhail et al., 2018). This was done at Oral Radiology department, Faculty of dentistry, Ain Shams University. Two radiographic images were obtained for each mandible.

Images brightness and contrast were adjusted, then bone density was measured along a line passing through molar area and perpendicular to a line tangent to inferior border of mandible (Fig. 1). Bone density was measured three times taking an average of readings. Data were collected and statistically analyzed.

2.5. Histological assessment

Molar areas of mandibles were decalcified by 12% EDTA solution. Half of mandibles were processed and sectioned mesiodistally while the other half were sectioned buccolingually. Mesiodistal sections were stained with Hematoxylin and Eosin.
(H&E) at Oral Biology department laboratory (Bancroft et al., 2013) and Masson Trichrome (MT) in a private laboratory (Bancroft and Gamble, 2008). MT was used to demonstrate newly formed immature blue stained collagen fibers in contrast to old mature red stained fibers. Buccolingual sections were stained with H&E. Sections were examined by light microscope (Olympus model: BX60F5 – Olympus optical company. Limited – Japan) with different magnifications at Oral Biology department, faculty of Dentistry, Ain Shams University.

2.6. Histomorphometric study

2.6.1. Buccal and lingual plates thickness

Buccolingual sections of first molar were used for measuring the cortical plates thickness. Three readings of mid-root area were taken from both buccal and lingual plates of each specimen. Mean values were calculated and used in histomorphometric analysis.

2.6.2. Masson Trichrome stain

MT-stained mesiodistal sections were used for measuring area percentage of newly formed immature collagen. Three fields were selected from each section x200 that were measured and analyzed by Image J v. 1.43u—National Institutes of Health, Bethesda, MD, USA.

2.6.3. Statistical analysis

Recorded data were analyzed using statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented as mean ± standard deviation and ranges. A one-way analysis of variance (ANOVA) was used when comparing between more than two means. Post Hoc test: Tukey’s test was used for multiple comparisons between different variables. Confidence interval was set to 95% and margin of error accepted was set to 5%. So, p-value was considered significant as the following: Probability P-value < 0.001 as highly significant, P-value < 0.05 as significant and P-value > 0.05 as insignificant.

3. Results

3.1. H and E results

Control group showed smooth alveolar margin and normal sized marrow cavities (Fig. 2A). Higher magnification showed plump osteoblasts while others appeared flattened. Osteocytes appeared normal and few osteoclasts could be seen (Fig. 2B). Osteoporotic group showed irregular alveolar margin with apparently enlarged marrow cavities (Fig. 2C). By Higher magnification, most regions revealed most osteoblasts are flattened. Some osteocytes lacunae

Fig. 1 Measuring bone density using Digora Software: Images brightness and contrast were first adjusted, then bone density was measured along a line passing through the molar area and perpendicular to a line tangent to the inferior border of the mandible.
appeared widened; few were empty while most appeared normal (Fig. 2D). Many osteoclasts were detected (Fig. 2E). Rutin group showed slightly undulating alveolar margin and normal sized marrow cavities (Fig. 2F). Higher magnification presented some plump osteoblasts lining osteoid and few flat osteoblasts. Osteocytes appeared normal (Fig. 2G). Few regions of few sections showed many osteoclasts (Fig. 2H).
3.2. Histochemical results (MT)

Control group revealed apparently equal areas of mature and immature collagen (Fig. 3A). Osteoporotic group showed an increase in mature bone regions than immature one (Fig. 3B). Rutin treated group expressed wide immature bone areas than mature bone (Fig. 3C).

3.3. Statistical results

3.3.1. Bone density

Bone density of osteoporosis group was highly significantly decreased when compared to both control and Rutin treated groups. While, Rutin treated group showed insignificant statistical difference when compared to control group (Table 1A and Fig. 4A).

3.3.2. Thickness of buccal and lingual cortical plates

In osteoporosis group, it was significantly decreased when compared to both control and Rutin treated groups. While, Rutin hydrate group showed insignificant statistical difference when compared to control (Table 1B and 1C, Fig. 4B and 4C).

3.3.3. Area percentage of newly formed collagen

In osteoporosis group, it was significantly decreased when compared to both control and Rutin treated groups. While, Rutin hydrate group showed insignificant statistical increase when compared to control (Table 1D, Fig. 4D).

4. Discussion

Osteoporosis is a skeletal disorder characterized by compromised bone strength. Bone quality could be assessed by evalu-
ating bone density. In the present work bone density was assessed radiologically via digital evaluation of bone samples radiographs (Khojastehpour et al., 2013). One month duration of methylprednisolone administration was selected to mimic GC chronic administration in human. Rutin hydrate used showed protective effect through its antioxidant and anti-inflammatory properties (Aktas et al., 2017). Male rats were used to rule out estrogen effect, as it is the most important hormone for maintaining normal bone turnover (Comelekoglu et al., 2007). H&E stain was used as it has been always considered the gold standard in histological assessment of different tissues (Rentsch et al., 2014). Thickness of Buccal and lingual cortical plates were selected as animal studies have shown link between systemic osteoporosis and bone loss in jaws (Chatterjee et al., 2021). To explore bone reparative capacity of, MT was used to evaluate collagen production and new bone formation.

The chief oral manifestations of osteoporosis are connected to alveolar ridge reduction, increased mandible and maxilla bone porosity, periodontal changes, thin trabeculae of bone and reduced maxillary bone mass and density (Watanabe et al., 2021). Mandibular density estimation is a simple and useful method in detecting decreased skeletal bone mineral density (BMD) (Pavicin et al., 2014).

In the current study, bone density in osteoporosis group was significantly decreased when compared to control. This agrees with Pavicin et al., 2014 who concluded that using mandibular density, is useful to recognize patients who should be referred for osteoporosis evaluation. Rutin hydrate treated group showed statistically higher mean bone density value.

Fig. 4  Bar charts comparing between groups according to: 4A: bone density. 4B: thickness of buccal cortical plate. 4C: thickness of lingual cortical plate. 4D: area percentage of newly formed collagen.
In the present study, H and E-stained sections of osteoporotic group showed irregular alveolar margin was with apparently enlarged narrow cavities. This is in accordance with Derakhshanian et al., 2013 who reported a decrease in trabecular thickness of femur in GC-treated rats. Abdel Fattah et al., 2019, documented that in GCs group alveolar bone of mandible in rats showed relatively irregular outline. Bone trabeculae appeared thin surrounding wide marrow cavities. In addition, Li et al., 2021 documented that bone trabeculae of mice tibia in prednisolone treated group were sporadically arranged with reduced number of trabeculae than in control.

In current study, many osteoclasts were detected in osteoporotic group. This is in accordance with Abdel Fattah et al., 2019 who detected many osteoclasts, indicating dominant bone resorption in GCs group. They also documented buccal cortical plate in same group revealed surface roughness and porosity with marked resorption and interruption of bone surface. Moreover, Li et al., 2021 reported by immunohistochemistry increased osteoblasts and osteoclasts number on tibial trabecular surface in prednisolone treated group more than that in control. They suggested that GCI osteoporosis showed high bone turnover with greater osteoblastic and osteoclastic activities, but the latter far exceeded the former.

Glucocorticoids increase bone resorption; by decreasing Osteoprotegerin expression, increasing receptor of activator of NF-kappa b ligand (RANKL) and reactive oxygen species (ROS). Glucocorticoids can prolong osteoclasts life span (Komori, 2016).

In osteoporosis group, most regions revealed most osteoblasts are flattened. This is in parallel with Derakhshanian et al., 2013 who reported a significant decrease in osteoblasts number in GC-treated rats. Furthermore, Abdel Fattah et al., 2019, observed lack or break of surface osteoblasts in GCs group.

In our study, some lacunae of osteocytes appeared widened; few were empty, while most appeared normal. This agrees with Kasem et al., 2016 who documented that upon comparing osteocyte number, there was a significant decrease between osteoporosis and control groups.

Feng et al., 2014 documented that GCs inhibit mesenchymal precursor cells differentiation into osteoblasts. Komori, 2016 reported that GCs stimulate osteoblast and osteocyte apoptosis through increasing pro-apoptotic molecules, ROS and endoplasmic reticulum stress and by suppressing the Wingless/Inte- grated/β-catenin pathway which is important for osteoblastic differentiation; thus, decreasing osteoblastogenesis.

In our study, when compared to control, osteoporosis group showed statistically significant decrease in buccal and lingual cortical plates thickness. This agrees with Derakhshanian et al., 2013 and Kasem et al., 2016 who reported a decrease in femur cortical thickness of osteoporosis group compared to control.

In our study, Rutin hydrate was able to restore bone mineral density. This is in agreement with Wang et al., 2017 who reported that rutin administration increased femur bone mineral density of ovarectomized rats.

In H and E-stained sections of Rutin hydrate group, alveolar margin was slightly undulating and marrow cavities appeared with normal sizes. Some plump osteoblasts lining osteoid, few flat osteoblasts and osteocytes appeared normal. It has been concluded by Horcajada-Molteni et al., 2000 that adding 0.25% of rutin to ovarectomized rats’ diet could inhibit trabecular bone loss of femur, by decreasing resorption and increasing osteoblastic activity. This leaded to increased femoral strength.

Moreover, it has been reported by Kyung et al., 2008 that rutin was able to significantly decrease the RANKL-induced formation of tartrate resistant acid phosphatase positive osteoclasts. They concluded that Rutin ability to inhibit osteoclastogenesis might result from ROS and tumor necrosis factor-α (TNF-α) decrease due to inhibition of RANKL-induced nuclear factor kappa-B activation. Also, Lee et al., 2020 documented that Rutin improved bone histomorphometric values through the decrease in osteoclastic activity with suggestion of new bone formation in ovarectomized mice. In our study, Rutin hydrate treated group showed decrease in osteoclasts except for few regions of few sections with many osteoclasts. This might indicate active bone remodeling due to Rutin hydrate administration.

In our study, the Rutin hydrate group showed statistically significant increase in buccal and lingual cortical plates thickness when compared to osteoporotic group. This agrees with Wang et al., 2017 who found that rutin could significantly enhance average femur trabecular bone thickness in ovarectomized rats. In their study, Rutin inverted elevated levels of interleukin-6, interferon-γ and TNF-α in ovarectomized rats.

Histochemical examination of osteoporotic group of current study, showed an increase in regions of mature bone than immature one. Statistical analysis revealed the area percentage of newly formed collagen in osteoporosis group was significantly decreased when compared to control. This could be attributed to that GCs have the ability to inhibit prostaglandins production like prostaglandin-E2 which can normally stimulate collagen and non-collagenous proteins synthesis (Raisz, 1999). Our results are in parallel with Sabry et al., 2021 who detected obvious reduction of blush-stained collagen content in bone matrix of ovarectomized rats.

Rutin hydrate treated group expressed wide areas of immature bone compared to those of mature bone. Statistical analysis showed that area percentage of newly formed collagen was significantly increased when compared to osteoporosis group and was statistically insignificant when compared to control group. This could be explained by Hyun et al., 2014 who documented that Rutin at a dose 25 µg/ml was able to increase collagenogenesis of human osteoblast-like MG-63 cells in vitro. The authors also reported the ability of rutin to enhance proliferation and differentiation of osteoblastic MG-63 cells. They declared that since collagen is considered as a main matrix component in bone, the increase in collagen content mirrors osteoblastic maturation.

5. Conclusions

The use of Rutin Hydrate was able to modify the radiological and histological picture of osteoporotic alveolar bone. This was achieved by the ability of Rutin Hydrate to increase bone

(159.62 ± 28.68A) when compared to osteoporotic group (110.37 ± 8.13B).
density, preserves cortical plates thickness and enhances new collagen formation. Further investigations are recommended to evaluate its effect with higher doses and longer durations.

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**Ethical statement**

The experiment was performed according to the guidelines of Ethics Committee, Faculty of Dentistry, Ain Shams University. The ethical approval number is (FDASU-IR022215).

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**References**

Abdel Fattah, H.S., El Masry, N.A., Kawana, K.Y., Khalil, N.M., 2019. Effect of bisphosphonates on the alveolar bone of rats with glucocorticoids induced osteoporosis. Alexandria Dental J. 4 (4), 65–70.

Akesson, K., 2003. New approaches to pharmacological treatment of Osteoporosis. Bull. World Health Organ. 81, 657–664.

Aktas, M.S., Kandemir, F.M., Özkaraç, M., Hanedan, B., Kirbas, A., 2017. Protective Effects of Rutin on Acute Lung Injury Induced by Oleic Acid in Rats. Kfks univ vet fark derg. 23 (3), 445–451.

Bancroft, J., Gamble, M., 2008. Connective Tissues and Stains in Theory and Practice of Histological Techniques. Churchill Livingstone, pp. 135–160.

Bancroft, J.D., Suvarna, K., Layton, C., 2013. Bancroft’s theory and practice of histological techniques. Elsevier, Churchill Livingstone.

Canalis, E., Mazziotti, G., Giustina, A., Bilezikian, J.P., 2007. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int. 18 (10), 1319–1328.

Chatterjee, M., Faot, F., Correa, C., Kerckhofs, J., Vandamme, K., 2013. Histological Effect of Bisphosphonate, Vitamin D and Olive Oil on Glucocorticoid Induced Osteoporosis (Gio) in Albino Rat. Egyptian J. Hospital Med. 65, 699–708.

Khojastehpour, L., Mogharrabi, S., Dabbaghmanesh, M.H., Nasrabadi, N., 2013. Comparison of the mandibular bone densitometry measurement between normal, osteopenic and osteoporotic postmenopausal women. J. Dentistry, Tehran Univ. Med. Sci. 10 (3), 203–209.

Kornori, T., 2016. Glucocorticoid signaling and bone biology. Horm. Metab. Res. 48, 755–763.

Kuang, T.W., Lee, J.E., Shin, H.H., Choi, H.S., 2008. Rutin inhibits osteoclast formation by decreasing reactive oxygen species and TNF-α by inhibiting activation of NF-KB. Exp. Mol. Med. 40 (1), 52–58.

Lee, H.H., Jang, J.W., Lee, J.K., Park, C.K., 2020. Rutin Improves Bone Histomorphometric Values by Reduction of Osteoclastic Activity in Osteoporosis Mouse Model Induced by Bilateral Ovariectomy. J. Korean Neurosurg. Soc. 63 (4), 433–443.

Li, C., Yang, P., Liu, B., Bu, J., Liu, H., Guo, J., Hasegawa, T., Shi, H., Li, M., 2021. Prednisolone induces osteocytes apoptosis by promoting Notum expression and inhibiting PI3K/AKT/GSK3β–β-catenin pathway. J. Mol. Histol. 52, 1081–1095.

Mahmoud, A.M., Hernandez Bautista, R.J., Sandhu, M.A., Hussein, O.E., 2019. Beneficial effects of citrus flavonoids on cardiovascular and metabolic health. Oxidative Medicine and Cellular Longevity, Article, p. 5484138.

Martin, R.M., Correa, P.H.S., 2010. Bone quality and osteoporosis therapy. Arq. Bras. Endocrinol. Metab. 54 (2), 186–199.

Mikhail, F.F., El-Din, M., Ibrahim, T., Zekry, K., Nemat, A., Nasry, S., 2018. Effect of Laser Therapy on the Osseointegration of Immediately Loaded Dental Implants in Patients under Vitamin C, Omega-3 and Calcium Therapy. Open Access Maced. J. Med. Sci. 6 (8), 1468–1474.

Nash, J.J., Nash, A.G., Leach, M.E., Poetker, D.M., 2011. Medical malpractice and corticosteroid use. Otolaryngol. Head Neck Surg. 144 (1), 10–15.

Nema, N., Bairagi, S.M., 2017. The In-Vivo Effects of Caffeine and Rutin Combination on Caffeine Intoxication in Rodent Model. Acta Sci. Pharm. Sci. 1 (1), 20–24.

Pavicin, S.I., Dumanic, J., Jukic, T., Badel, T., Badanjak, A., 2014. Digital orthopantomograms in osteoporosis detection: mandibular density and mandibular radiographic indices as skeletal BMD predictors. Dentomaxillofac. Radiol. 43 (7), 20130366.

Peng, C.H., Lin, W.Y., Yeh, K.T., Chen, I.H., Wu, W.T., Lin, M.D., 2021. The molecular etiology and treatment of glucocorticoid-induced osteoporosis. Tzu Chi. Med. J. 33 (3), 212–223.

Raisz, L.G., 1999. Prostaglandins and bone: physiology and pathophysiology. Osteoarthr. Cartilage. 7, 419–421.

Rentsch, C., Schneiders, W., Manthey, S., Rentsch, B., Rammelt, S., 2014. Comprehensive histological evaluation of bone implants. Biomatter. 4 (1), e27993.

Saby, M., Mostafia, S., Kamar, S., Rashid, L., Estaphan, S., 2021. The cross-talk between matrix metalloproteinase-9, RANKL/OPG system and cardiovascular risk factors in ovariectomized rat model of postmenopausal osteoporosis. PLoS ONE 16, (10) e0258254.
Steinbuch, M., Youket, T.E., Cohen, S., 2004. Oral glucocorticoid use is associated with an increased risk of fracture. Osteoporos. Int. 15, 323–328.
Wang, Q.L., Huo, X.C., Wang, J.H., Wang, D.P., Zhu, Q.L., Liu, B., Xu, L.L., 2017. Rutin prevents the ovariectomy-induced osteoporosis in rats. Eur. Rev. Med. Pharmacol. Sci. 21, 1911–1917.
Wang, T., Han, C., Tian, P., Li, P.F., Ma, X.L., 2018. Role of Teriparatide in Glucocorticoid-induced Osteoporosis through Regulating Cellular Reactive Oxygen Species. Orthop. Surg. 10 (2), 152–159.
Watanabe, P.C., Rodrigues, G. A., Azenha, M. R., Ribeiro, M.C., Filho, E.D.S., Vieira, R.A., Bottacin, F.S., 2021. Bone Quality of the Dento-Maxillofacial Complex and Osteoporosis. Opportunistic Radiographic Interpretation. In: Osteoporosis-Recent Advances, New Perspectives and Applications. Mar 12. IntechOpen. 96487.
Weinstein, R.S., 2012. Glucocorticoid-induced osteoporosis and osteonecrosis. Endocrinol. Metab. Clin. North Am. 41 (3), 595–611.