



ORIGINAL ARTICLE

Assessment of bone marrow-derived mesenchymal stem cells capacity for odontogenic differentiation and dentin regeneration in methimazole-treated albino rats (Light microscopic Study)



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KEYWORD

Dentin Regeneration;
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Abstract *Background:* Methimazole is an antithyroid drug. It has side effects on many tissues. Bone marrow-derived mesenchymal stem cells (BM-MSCs) are promising in the field of tissue regeneration.

Objective: To investigate the capacity of BM-MSCs on odontogenic differentiation and dentin regeneration at different time intervals in methimazole treated rats.

Methods: Twenty-eight male albino rats were classified as: **Group I:** got distilled water. **Group II:** obtained therapeutic dosage of methimazole as pro-drug “Neo-Mercazole®”. **Group III:** received methimazole then solitary injection of BM-MSCs at day 21. **Group IV:** obtained methimazole and single injection of BM-MSCs at the beginning of the experiment. Light microscope was used to examine specimens. Recently formed collagen and β -catenin-immunoreactivity area% were appraised histomorphometrically and statistically.

Results: Histological examination of odontoblasts and dentin illustrated normal structure in Group I and nearly normal features in Group IV. Group II demonstrated discontinuation of odontoblastic layer and areas of different stainability in dentin. Group III showed an evidently wide layer of odontoblast-like cells and distinct dentinal tubules. Masson’s trichrome results of dentin in Groups I & IV showed apparently equal areas of new and old collagen. Group II illustrated old collagen mainly. Group III explored new collagen only. β -catenin-immunoreactivity was strong in Groups I & IV, mild in Group II and moderate in Group III. Statistical results revealed that the highest mean of newly formed collagen area% was in Group III, followed by Group I, Group IV

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then Group II respectively. Regarding β -catenin-immunoreactivity area%, the highest mean was recorded in Group I, subsequently Group IV, next Group III then Group II.

Conclusions: Methimazole has destructive consequences. BM-MSCs have a time-based increased capacity for odontogenic differentiation and regeneration of dentin.

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1. Introduction

Odontoblasts are specialized post-mitotic cells that belong to pulp cells and act as nociceptors along with their main role in dentin formation (Kawashima and Okiji, 2016).

β -catenin is expressed in nucleus and cytoplasm of odontoblasts. It regulates transcriptional factor “Runt-related transcription factor 2” which is master regulator of odontoblast differentiation. Also, β -catenin is fundamental for expression of dentin sialophosphoprotein, dentin matrix protein 1, alkaline phosphatase and bone sialoprotein which are responsible for dentin matrix formation and mineralization (Han et al., 2014; Miyashita et al., 2017).

Methimazole “antithyroid drug” is active metabolite of carbimazole. Some studies recorded declination in thyroid hormones at second, fourth and sixth week post-treatment by methimazole (Choi and Jee, 2015; Sakr et al., 2012; Tataru et al., 2017).

Many adverse effects of methimazole were recorded such as impairment of calcium and protein metabolism as well as declined concentration of different amino acids (Tataru et al., 2017). Moreover, methimazole negatively affects expression of different markers responsible for survival, proliferation and function of different hard tissue forming cells like osteoblasts and odontoblasts (Aghajanian et al., 2017; White et al., 2016).

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are very important population among adult stem cells due to their promising outcomes (Aghajanian et al., 2017; Saraswathi and Saravanakumar, 2010). BM-MSCs can differentiate into odontogenic lineage and significantly participate to odontoblast population by expressing odontoblast-specific marker “dentin sialoprotein” (Zheng et al., 2019).

From this perspective, this study was performed to explore histological structure of odontoblasts and dentin along the course of methimazole administration and potential effect of BM-MSCs at disparate time intervals.

2. Materials and methods

Ethical statement

Experiment steps were approved by Research Ethics Committee at Future University in Egypt, (FUE.REC (22)/11–2020).

2.1. Isolation and culture of BM-MSCs

Isolation and culture of BM-MSCs were performed as mentioned in our previous study (Rabea, 2020).

2.2. Animals and study design

A total of 28 adult healthy male albino rats weighing 180–200gm were purchased and housed at animal breeding colony at Faculty of Medicine-Cairo University.

After acclimatization for 1 week, categorization of rats randomly into 4 equal groups was done.

Group I: received distilled water “vehicle” (5 ml/kg b.wt/day) for 42 days by intra-gastric intubation (Nampoothiri et al., 2015).

Group II: received methimazole in form of pro-drug “Neo-Mercazole® 5 mg” (Amdipharm Mercury, Australia) through intra-gastric intubation. Therapeutic dosage (1.35 mg/kg b.wt/once/day) was deliquesced in distilled water (5 ml) and applied for 42 days (Sakr et al., 2017).

Group III: received methimazole as formerly described and at day 21, each animal was injected once at tail vein by BM-MSCs (1×10^7) in phosphate buffered saline (1 ml) under anesthesia via inhalation of ether (Elsaadany et al., 2017).

Group IV: received methimazole as described formerly. Concurrently at starting of methimazole administration every rat got solitary BM-MSCs injection as mentioned previously (Elsaadany et al., 2017).

2.3. Sacrifice of animals

Over dosage of ketamine was applied to euthanize all rats (Shredah and El-Sakhawy, 2014).

2.4. Specimens preparation and staining

To examine mandibular molars in the jaws, the two halves of mandible were dissected sagittally, fixed, decalcified and sections were prepared for hematoxylin and eosin (H&E) and Masson’s trichrome stain (Arima et al., 2019; Bancroft, 2002; Bancroft and Layton, 2013; Shredah and El-Sakhawy, 2014), as well as for β -catenin immunolocalization as mentioned previously in our study (Rabea, 2020).

Examination of tissues was done using light microscope (Olympus® BX 60, Tokyo, Japan). Both, odontoblasts and dentin were photographed at 400 \times magnification.

Anti- β -catenin antibody was chosen due to great role of β -catenin in differentiation of odontoblasts and dentin formation (Han et al., 2014; Miyashita et al., 2017).

2.5. Histomorphometric analysis

Histomorphometric analysis was carried out by using image J software. For measuring area% of newly formed collagen and β -catenin-immunoreactivity; 7 images from 7 different fields in each slide of Masson’s trichrome and immunolabeled sections were used. Images were transformed into grey delineated one and areas of interest were masked by a blue binary color.

2.6. Statistical analysis

One-way ANOVA test was chosen to compare different studied groups followed by post hoc test “Least Significant Differ-

ence". Probability value (P-value) < 0.001 designates highly significant results whereas; P-value < 0.05 points to significant results. Utilized software was Statistical Package for the Social Sciences 20 (SPSS® Inc., Chicago, USA).

3. Results

3.1. Histological results

3.1.1. Group I

Histological inspection of Group I showed normal structure of odontoblasts and dentin. Odontoblasts formed a continuous layer that lined pulp periphery. They exhibited tall columnar shape with polarized cell bodies (oval nuclei were basally located). Their processes were extended into dentin. Adjacent to odontoblasts' cell bodies; there was a definite apparently wide layer of predentin at pulpal surface of dentin. Dentin showed well-defined parallel dentinal tubules and intertubular dentin (Fig. 1a).

3.1.2. Group II

Examination of Group II histological sections showed discontinuation of odontoblastic layer. Some odontoblasts were detached from dentin and became desquamated in pulp tissue. Most of odontoblasts apparently lost their cell height and appeared shrunken. Some cells had pyknotic and hyperchromatic nuclei. Obviously very narrow, discontinued and ill-defined layer of predentin was observed. Dentin represented ill-defined dentinal tubules and intertubular dentin as well as areas of different stainability (Fig. 1b).

3.1.3. Group III

Examination of Group III histologically illustrated perceptibly wide layer of pre-odontoblasts. Continuous evidently wide layer of apparently numerous odontoblast-like cells was identified; some cells were tall columnar with basally located oval nuclei while others were cuboidal. Cells' processes were extended into dentin. Ostensibly narrow but well-defined continuous layer of predentin was detected. Distinct dentinal tubules and intertubular dentin were observed (Fig. 1c).

3.1.4. Group IV

Histological examination of Group IV demonstrated almost normal histological structure of both odontoblast-like cells and dentin. Pre-odontoblastic layer and incessant layer of odontoblast-like cells were spotted. Odontoblast-like cell bodies were tall columnar and displayed basal oval nuclei while their processes were protracted into dentin. Allegedly wide, continuous and distinct predentin layer was present. Well-demarcated parallel dentinal tubules and intertubular dentin were noticed in dentin (Fig. 1d).

3.2. Masson's trichrome results

Dentin in all groups was examined. Group I & Group IV exemplified some areas of newly formed collagen and apparently equivalent areas of old collagen (Fig. 2a and d). On the other hand, Group II showed mainly old collagen except for evidently very narrow predentin layer and peritubular dentin in perceptibly few dentinal tubules which presented new colla-

gen (Fig. 2b). However, only newly formed collagen was seen in Group III (Fig. 2c).

3.3. Immunohistochemical results

Strong β -catenin-immunoreactivity was detected in odontoblasts and odontoblast-like cells (nuclear and cytoplasmic) as well as dentin of Groups I & IV respectively (Fig. 3a and d). Group II illustrated mild nuclear and cytoplasmic β -catenin-immunoreactivity in odontoblasts. Dentin revealed mild β -catenin-immunoreactivity (Fig. 3b). Moderate β -catenin-immunoreactivity was exemplified in odontoblast-like cells (nuclear and cytoplasmic) as well as dentin of Group III (Fig. 3c).

3.4. Statistical results

There was statistically high significant difference between studied groups regarding area% of new collagen and β -catenin-immunoreactivity as follows:

3.4.1. Newly formed collagen area%

Studied groups were arranged in descending order as follows: Groups III, I, IV then II respectively (Table 1 and Fig. 4a).

3.4.2. β -catenin-immunoreactivity area%

The descending order of studied groups was: Groups I, IV, III then II respectively (Table 2 and Fig. 4b).

4. Discussion

Methimazole has many undesired effects as it attenuates activities of antioxidant enzymes which results in creation of oxidative stress condition that culminates by cellular damage (Al-Amoudi et al., 2016; Sakr et al., 2015).

In regenerative medicine BM-MSCs play important role not only due to their ability of differentiation among multiple lineages and self-renewal property but also, because they resist hypoxia-induced apoptosis and oxidative stress-induced senescence (El-Badawy et al., 2016).

Male albino rats were exploited in herein research to evade effect of female hormones (Souza et al., 2014).

In present research, ordinary histological features of odontoblasts and dentin recorded in results of Group I are in line with those of (Han et al., 2014).

Degenerative changes detected in Group II of this study could be attributed to enhancement effect of methimazole on production of tumor necrosis factor alpha through increasing of oxidative stress which in turn inhibits expression of bone morphogenetic protein-2 and bone morphogenetic protein-9 genes which have key role in odontoblastic differentiation (Bashandy, 2018; Wang et al., 2017; Yanget al., 2015). Also, oxidative stress promotion, down regulation of antioxidant enzymes activities, reactive oxygen and nitrogen species accumulation as well as lipid peroxidation reinforcing are consequences of methimazole intake that lead to damage of biomolecules like phospholipids, DNA and proteins (Sakr et al., 2015). Different stainability of dentin detected in Group II of herein study might be due to imbalance of minerals as an adverse outcome of methimazole on hard tissue mineralization

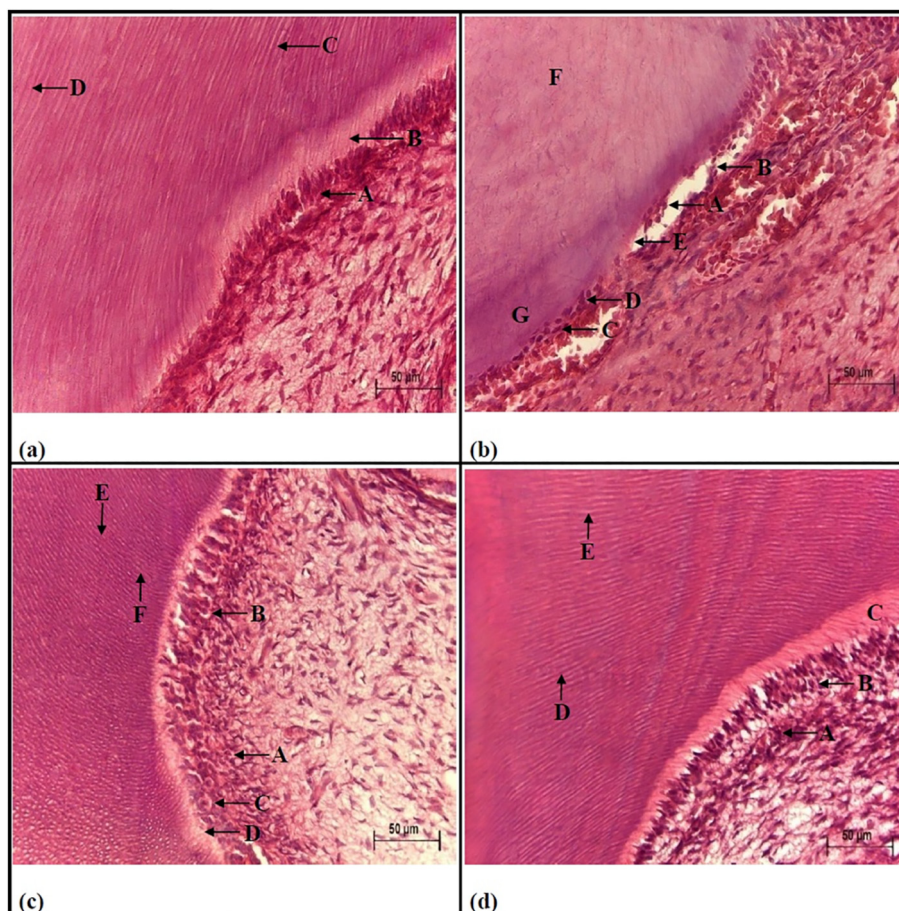


Fig. 1 H&E-stained sections: (a)- Group I showing: continuous layer of tall columnar odontoblasts with basal oval nuclei (A). Definite apparently wide layer of pre-dentin (B). Dentin with well-defined parallel dentinal tubules (C) and intertubular dentin (D). (b)- Group II showing: discontinued odontoblastic layer (A). Some detached and desquamated odontoblasts (B). Most of odontoblasts apparently lose their cell height and appear shrunken (C). Some cells have pyknotic and hyperchromatic nuclei (D). Obviously very narrow, discontinued and ill-defined layer of pre-dentin (E). Dentin with ill-defined dentinal tubules and intertubular dentin (F) as well as areas of different stainability (G). (c)- Group III showing: perceptibly wide layer of pre-odontoblasts (A). Continuous evidently wide layer of apparently numerous odontoblast-like cells with some cells have tall columnar shape and basally located oval nuclei (B) while others are cuboidal (C). Ostensibly narrow but well-defined continuous layer of pre-dentin (D). Distinct dentinal tubules (E) and intertubular dentin (F). (d)- Group IV showing: pre-odontoblastic layer (A). Incessant layer of tall columnar odontoblast-like cells with basal oval nuclei (B). Allegedly wide, continuous and distinct pre-dentin layer (C). Well-demarcated parallel dentinal tubules (D) and intertubular dentin (E), (x400).

(Ferretti et al., 1992). It was proved that total alkaline phosphatase as well as phosphorus and calcium levels can be reduced under influence of methimazole (Ben Amara et al., 2012). It is well known that alkaline phosphatase enzyme plays important role in formation and maintenance of dentin (Han et al., 2014). Additionally, methimazole adversely affects expression of dentin matrix protein 1 as well as transient receptor potential vanilloid type 4 “calcium ion channel” present in odontoblasts which leads to reduction of channel permeability and thus decreased influx of calcium ions (Aghajanian et al., 2017; White et al., 2016). Moreover, methimazole can reduce concentration of serum osteocalcin “dentin formation marker” by over 21% (Tatara et al., 2017; Zeichner-David, 2006).

Group III in current study revealed many reparative features which are in agreement with those of (Lei et al., 2013; Saraswathi and Saravanakumar, 2010). Our results could be clarified according to the fact that after 2 weeks of BM-MSCs administration *in vivo* some of them become differenti-

ated into odontoblast-like cells as they expressed odontoblast-specific marker “dentin sialoprotein” along with beginning of reparative tubular dentin formation (Chmilewsky et al., 2014; Lei et al., 2013). Moreover, oxidative stress activates cellular tumor antigen p53 which is principle for BM-MSCs self-renewal and stemness (Katagiri et al., 2016). Furthermore, BM-MSCs possess pro-survival/proliferative effects on resident pulp stem cells. In addition, BM-MSCs attenuate production of reactive oxygen and nitrogen species as well as interferon-gamma “inflammatory molecule” in accompaniment with secretion of antioxidants, anti-inflammatory mediators as well as growth factors which result in improvement of angiogenesis, cell survival and immune modulation (Chen et al., 2008; Iyer and Rojas, 2008; Kaneko et al., 2019; Mumaw et al., 2015; Uccelli et al., 2008).

More or less normal histological features were illustrated in Group IV of current research. This result could be explained according to Goldberg (2019) and Lei et al. (2013) who stated

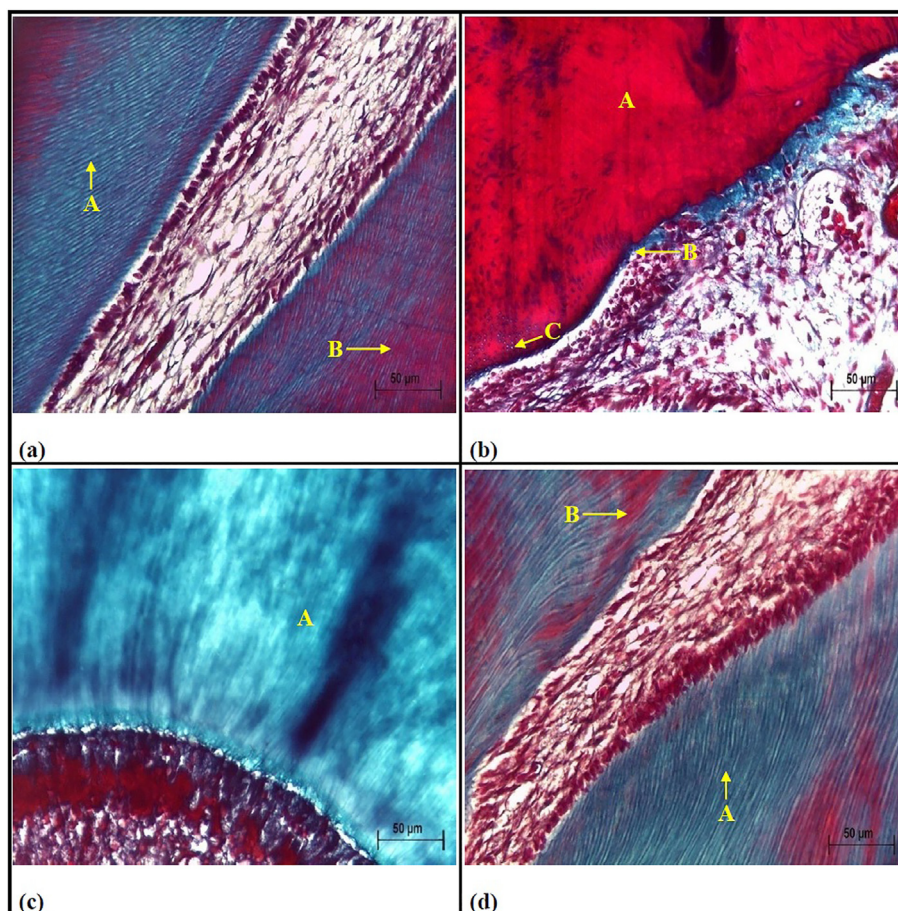


Fig. 2 Masson's trichrome-stained sections: (a)- Group I showing: dentin with some areas of newly formed collagen (A) and apparently equivalent areas of old collagen (B). (b)- Group II showing: mainly old collagen in dentin (A). New collagen in evidently very narrow predentin layer (B) and peritubular dentin in perceptibly few dentinal tubules (C). (c)- Group III showing: only newly formed collagen in dentin (A). (d)- Group IV showing: some areas of newly formed collagen (A) and apparently equivalent areas of old collagen (B) in dentin, (x400).

that after 6 weeks of BM-MSCs administration the number of differentiated cells increases greatly and the differentiated cells become fully matured and produce reactionary dentin which has structure close to that of secondary regular dentin.

Masson's trichrome fallout and statistical results of new collagen area% in Group I of the current study might be accredited to continuous process of dentin formation, as rate of secondary dentin formation in erupted tooth is $0.5 \mu\text{m}/\text{day}$ (Bleicher, 2014). Predentin as well as newly formed secondary dentin contain mainly newly synthesized immature collagen (type III), (Goldberg et al., 2012). Additionally, mature dentin demonstrates that 90% of its organic component is mature collagen (type I) and the remaining is in form of immature collagen (type III) in addition to collagen type V as well as non-collagenous proteins (Kawashima and Okiji, 2016).

In this research, Group II fallouts of Masson's trichrome and statistics of new collagen area% might be attributed to reinforcement effect of methimazole on matrix metalloproteinases formation that cleave collagen I, II and III (Bashandy, 2018; Yaoita, 2019).

It was reported that some of BM-MSCs become differentiated while others possess regenerative function through their

paracrine effect (Wang et al., 2015). Therefore, results of both Masson's trichrome and statistics of new collagen area% of Group III in present research might be ascribed to regenerative action of BM-MSCs as they exhibit pro-survival/proliferative effects on tenant pulp stem cells by means of their paracrine effect which includes secretion of cytokines and growth factors, in addition to modulation of inflammation (Chen et al., 2008; Iyer and Rojas, 2008; Kaneko et al., 2019; Mumaw et al., 2015; Uccelli et al., 2008). Moreover, BM-MSCs differentiate into odontoblast-like cells and differentiated cells express odontoblast-specific marker "dentin sialoprotein" in conjunction with enhancement of dentinogenesis and initiation of reparative tubular dentin formation. This newly formed dentin contains mainly immature collagen (type III), (Chmielewsky et al., 2014; Goldberg, 2019; Lei et al., 2013).

Both of Masson's trichrome and statistics results of new collagen area% of Group IV in this research could be clarified by dramatic increase of differentiated cells which also become fully matured after 6 weeks of BM-MSCs administration as well as reactionary dentin formation in which its structure closely resembles that of secondary regular dentin which contains primarily mature collagen (type I) while immature collagen

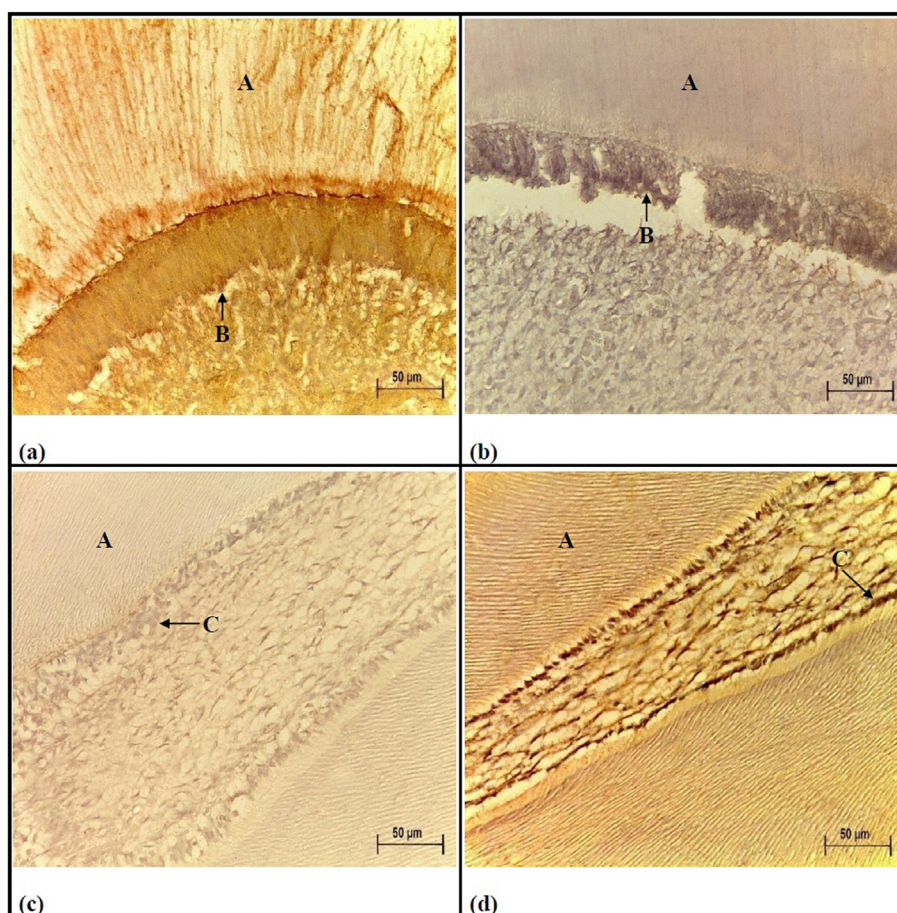


Fig. 3 β -catenin-stained sections of studied groups, displaying different immunoreactivity patterns in dentin (A), different patterns of nuclear and cytoplasmic immunoreactivity in odontoblasts (B) and odontoblast-like cells (C). (a)- Group I presenting: strong immunoreactivity. (b)- Group II exhibiting: mild immunoreactivity. (c)- Group III revealing: moderate immunoreactivity. (d)- Group IV illustrating: strong immunoreactivity, (x400).

Table 1 Displaying the range values, mean \pm SD, ANOVA and Least Significant Difference post hoc results of newly formed collagen area% in different studied groups.

Newly formed collagen area%	Group I	Group II	Group III	Group IV	ANOVA	p-value
Mean \pm SD	44.62 \pm 0.09	2.91 \pm 0.08 ^a	96.99 \pm 0.08 ^{ab}	42.60 \pm 0.09 ^{abc}	224.669	< 0.001**
Range	44.5–44.7	2.8–3	96.9–97.1	42.5–42.7		

Superscript letters according to Least Significant Difference post hoc test designate; a: significant difference versus Group I; b: significant difference versus Group II; c: significant difference versus Group III.

** : High significance at p-value < 0.001

(type III) is restricted to predentin and newly formed dentin (Goldberg, 2019; Goldberg et al., 2012; Kawashima and Okiji, 2016; Lei et al., 2013).

In herein study, results of immunohistochemical examination and statistical results of β -catenin-immunoreactivity area % of Group I might be attributed to important role of Wnt/ β -catenin signaling pathway in controlling differentiation of odontoblasts and formation of dentin matrix as well as its mineralization (Han et al., 2014; Miyashita et al., 2017).

Results of immunohistochemical examination and statistical results of area% of β -catenin-immunoreactivity recorded

in Group II of this study could be ascribed to oxidative stress condition created by methimazole that impedes Wnt/ β -catenin signaling pathway. Accordingly these events inhibit odontoblasts differentiation, reduce their viability, down regulate production of dentin matrix and interfere with its mineralization (Bashandy, 2018; Han et al., 2014; Miyashita et al., 2017; Ramazzotti et al., 2019).

In this research, results of immunohistochemical examination and statistical results of β -catenin-immunoreactivity area % illustrated in Group III might be explained by the fact that secretion of antioxidant molecules as well as angiogenic factors

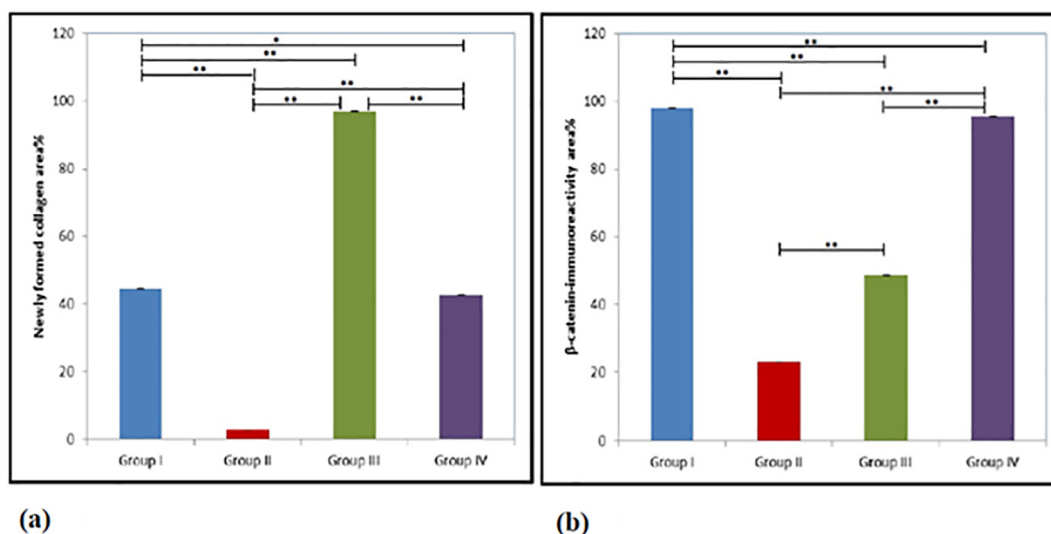


Fig. 4 (a)- Bar chart showing values of means and SD for newly formed collagen area% in all studied groups. * $P < 0.05$, ** $P < 0.001$ (b)- Bar chart displaying values of means and SD for β -catenin-immunoreactivity area% in all studied groups. ** $P < 0.001$.

Table 2 Presenting the range values, mean \pm SD, ANOVA and Least Significant Difference post hoc results of β -catenin-immunoreactivity area% in different studied groups.

β -catenin-immunoreactivity area%	Group I	Group II	Group III	Group IV	ANOVA	p-value
Mean \pm SD	97.91 \pm 0.07	22.91 \pm 0.08 ^a	48.61 \pm 0.08 ^{ab}	95.51 \pm 0.09 ^{abc}	116.397	< 0.001**
Range	97.8–98	22.8–23	48.5–48.7	95.4–95.6		

Superscript letters according to Least Significant Difference post hoc test designate; a: significant difference versus Group I; b: significant difference versus Group II; c: significant difference versus Group III.

** : High significance at p-value < 0.001.

by BM-MSCs contributes to regeneration/repair. In addition, stemness of BM-MSCs is maintained during regeneration of tissues suffering from oxidative stress (Prockop, 2017). Moreover, throughout tissue regeneration stemness of BM-MSCs is accompanied by activation of many signaling pathways like Wnt/ β -catenin (Pal and Das, 2017). Wnt/ β -catenin signaling can influence BM-MSCs to differentiate into odontoblast-like cells (Goldberg, 2017; Miyashita et al., 2017).

In present research, results of immunohistochemical examination and statistical analysis of β -catenin-immunoreactivity area% of Group IV might be ascribed to different genes expression which exhibit biological functions like Wnt/ β -catenin signaling during early BM-MSCs stages of differentiation as well as up regulation of their expression during tissue regeneration and repair in time dependent manner (Arvidson et al., 2011; Goldberg, 2017; Liu et al., 2019).

More inquiries are needed for applying BM-MSCs strategy on patients receiving methimazole.

5. Conclusions

Methimazole has damaging impact not only on structure of odontoblasts and dentin but also on their homeostasis.

Over time, BM-MSCs have up-regulated competence to improve destruction induced by methimazole through odontogenic differentiation and dentin regeneration.

For clinical implication, conservative dental treatment for methimazole-treated patients must be with special care.

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CRedit authorship contribution statement

Amany A. Rabea: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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

Aghajanian, P., Xing, W., Cheng, S., Mohan, S., 2017. Epiphyseal bone formation occurs via thyroid hormone regulation of chondrocyte to osteoblast transdifferentiation. *Sci. Rep.* 7, 1–12.

- Al-Amoudi, W.M., Mahboub, F.A., Lamfon, H.A., Lamfon, N.A., 2016. Assessing induced effect of curcumin on methimazole hepatic damage in albino rats: a histological and histochemical study. *Canadian J. Pure Appl. Sci.* 10 (3), 3961–3969.
- Arima, M., Hasegawa, D., Yoshida, S., Mitarai, H., Tomokiyo, A., Hamano, S., Sugii, H., Wada, N., Maeda, H., 2019. R-spondin 2 promotes osteoblastic differentiation of immature human periodontal ligament cells through the Wnt/ β -catenin signaling pathway. *J. Periodont. Res.* 54, 143–153.
- Arvidson, K., Abdallah, B.M., Applegate, L.A., Baldini, N., Cenni, E., Gomez-Barrena, E., Granchi, D., Kassem, M., Konttinen, Y.T., Mustafa, K., Pioletti, D.P., Sillat, T., Finne-Wistrand, A., 2011. Bone regeneration and stem cells. *Regenerative Medicine Review Series. J. Cell. Mol. Med.* 15 (4), 718–746.
- Bancroft, J.D., 2002. Trichrome stains. In: Bancroft, J.D., Gamble, M. (Eds.), *Theory and Practice of Histological Techniques*. Churchill Livingstone, Philadelphia, pp. 162–175.
- Bancroft, J.D., Layton, C., 2013. The hematoxylin and eosin. In: Suvarna, S.K., Layton, C., Bancroft, J.D. (Eds.), *Theory and Practice of Histological Techniques*. Churchill Livingstone, Philadelphia, pp. 173–186.
- Bashandy, M.A., 2018. Role of rosemary (*Rosmarinus officinalis*) extract on carbimazole induced alteration in pancreas of adult male albino rat (histological, immuno-histochemical and ultrastructural study). *J. Am. Sci.* 14 (1), 110–124.
- Ben Amara, I., Troudi, A., Soudani, N., Guermazi, F., Zeghal, N., 2012. Toxicity of methimazole on femoral bone in suckling rats: alleviation by selenium. *Exp. Toxicol. Pathol.* 64 (3), 187–195.
- Bleicher, F., 2014. Odontoblast physiology. *Exp. Cell Res.* 325, 65–71.
- Chen, L., Tredget, E.E., Wu, P.Y., Wu, Y., 2008. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS ONE* 3 (4), 1–12.
- Chmilewsky, F., Jeanneau, C., Dejou, J., About, I., 2014. Sources of dentin-pulp regeneration signals and their modulation by the local microenvironment. *J. Endod.* 40 (4), 1–25.
- Choi, J., Jee, J.G., 2015. Repositioning of thiourea-containing drugs as tyrosinase inhibitors. *Int. J. Mol. Sci.* 16, 28534–28548.
- El-Badawy, A., Amer, M., Abdelbaset, R., Sherif, S.N., Abo-Elela, M., Ghallab, Y.H., Abdelhamid, H., Ismail, Y., El-Badri, N., 2016. Adipose stem cells display higher regenerative capacities and more adaptable electro-kinetic properties compared to bone marrow-derived mesenchymal stromal cells. *Sci. Rep.* 6, 1–11.
- Elsaadany, B., El Kholy, S., El Rouby, D., Rashed, L., Shouman, T., 2017. Effect of transplantation of bone marrow derived mesenchymal stem cells and platelets rich plasma on experimental model of radiation induced oral mucosal injury in albino rats. *Int. J. Dentistry* 2017, 1–9.
- Ferretti, J.L., Vbzquez, S.O., Delgado, C.I., Capozza, R., Cointry, G., 1992. Biphasic dose-response curves of cortisol effects on rat diaphyseal bone biomechanics. *Calcif. Tissue Int.* 50 (1), 49–54.
- Goldberg, M., 2017. Stem cells: tools for dental tissues regeneration. *J. Dent. Health Oral Disord. Ther.* 6(4), 111–116.
- Goldberg, M., 2019. Indirect versus direct pulp capping: reactionary versus reparative dentin. *J. Dent. Health Oral Disord. Ther.* 10 (1), 95–96.
- Goldberg, M., Kulkarni, A.B., Young, M., Boskey, A., 2012. Dentin: structure, composition and mineralization: the role of dentin ECM in dentin formation and mineralization. *Front. Biosci.* 3, 711–735.
- Han, N., Zheng, Y., Li, R., Li, X., Zhou, M., Niu, Y., Zhang, Q., 2014. β -catenin enhances odontoblastic differentiation of dental pulp cells through activation of Runx2. *PLoS ONE* 9 (2), 1–10.
- Iyer, S.S., Rojas, M., 2008. Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. *Expert Opin. Biol. Ther.* 8, 569–581.
- Kaneko, T., Sone, P.P., Zaw, S.Y.M., Sueyama, Y., Zaw, Z.C.T., Okada, Y., Murano, H., Gu, B., Okiji, T., 2019. *In vivo* fate of bone marrow mesenchymal stem cells implanted into rat pulpotomized molars. *Stem Cell Res.* 38, 1–6.
- Katagiri, H., Kushida, Y., Nojima, M., Kuroda, Y., Wakao, S., Ishida, K., et al, 2016. A Distinct subpopulation of bone marrow mesenchymal stem cells, muse cells, directly commit to the replacement of liver components. *Am. J. Transplant.* 16, 468–483.
- Kawashima, N., Okiji, T., 2016. Odontoblasts: specialized hard-tissue-forming cells in the dentin-pulp complex. *Congenital Anomalies* 56, 144–153.
- Lei, G., Yu, Y., Jiang, Y., Wang, S., Yan, M., Smith, A.J., Smith, G., Cooper, P.R., Tang, C., Zhang, G., Yu, J., 2013. Differentiation of BMMSCs into odontoblast-like Cells induced by natural dentine matrix. *Arch. Oral Biol.* 58 (7), 862–870.
- Liu, J., Ruan, J., Weir, M.D., Ren, K., Schneider, A., Wang, P., Oates, T.W., Chang, X., Xu, H.H.K., 2019. Periodontal bone-ligament-cementum regeneration via scaffolds and stem cells. *Cells* 8 (6), 1–24.
- Miyashita, S., El Motaz Bellah, A.N., Murakami, M., Iohara, K., Yamamoto, T., Horibe, H., Kurita, K., Takano-Yamamoto, T., Nakashima, M., 2017. Mechanical forces induce odontoblastic differentiation of mesenchymal stem cells on three-dimensional biomimetic scaffolds. *J. Tissue Eng. Regen. Med.* 11, 434–446.
- Mumaw, J.L., Schmiedt, C.W., Breidling, S., Sigmund, A., Norton, N. A., Thoreson, M., Peroni, J.F., Hurley, D.J., 2015. Feline mesenchymal stem cells and supernatant inhibit reactive oxygen species production in cultured feline neutrophils. *Res. Vet. Sci.* 103, 60–69.
- Nampoothiri, M., John, J., Kumar, N., Mudgal, J., Nampurath, G., Chamallamudi, M., 2015. Modulatory role of simvastatin against aluminium chloride-induced behavioural and biochemical changes in rats. *Behav. Neurol.* 2015, 1–9.
- Pal, B., Das, B., 2017. *In vitro* culture of naïve human bone marrow mesenchymal stem cells: a stemness based approach. *Front. Cell Dev. Biol.* 5, 1–19.
- Prockop, D.J., 2017. The exciting prospects of new therapies with mesenchymal stromal cells. *Cytotherapy* 19, 1–8.
- Rabea, A.A., 2020. Histological, Histochemical and Immunohistochemical Evaluation of the Role of Bone Marrow-Derived Mesenchymal Stem Cells on the Structure of Periodontal Tissues in Carbimazole-Treated Albino Rats. *Arch. Oral Biol.* 119, 1–10.
- Ramazzotti, G., Ratti, S., Fiume, R., Follo, M.Y., Billi, A.M., Rusciano, I., Obeng, E.O., Manzoli, L., Cocco, L., Faenza, I., 2019. Phosphoinositide 3 kinase signaling in human stem cells from reprogramming to differentiation: a tale in cytoplasmic and nuclear compartments. *Int. J. Mol. Sci.* 20, 1–11.
- Sakr, S.A., Abdel-Ghafar, F.R., Abo-El-Yazid, S.M., 2015. Selenium ameliorates carbimazole induced hepatotoxicity and oxidative stress in albino rats. *Journal of Coastal Life Medicine* 3 (2), 139–145.
- Sakr, S. A., Hassab ELnaby, S. E., Okdah, Y. A., El-Shabka, A. M., 2017. Impact of ginger aqueous extract on carbimazole induced testicular degenerative alterations and oxidative stress in albino rats. *J. Coast. Life Med.* 5(4), 167–173.
- Sakr, S.A., Mahran, H.A., Nofal, A.E., 2012. Effect of selenium on carbimazole induced histopathological and histochemical alterations in prostate in albino rats. *American J. of Medicine and Medical. Science* 2 (1), 5–11.
- Saraswathi, P., Saravanakumar, S., 2010. A simple method of tooth regeneration by bone marrow mesenchymal stem cells in albino rats. *Eur. J. Anat.* 14 (3), 121–126.
- Shredah, M., El-Sakhawy, M., 2014. Immunohistochemical expression of activated caspase-3 in the parotid salivary glands of rats after long administration of *Myristica fragrans*. *Int. J. Adv. Res.* 2 (12), 493–499.
- Souza, M.F., Couto-Pereira, N.S., Freese, L., Costa, P.A., Caletti, G., Bisognin, K.M., Nin, M.S., Gomez, R., Barros, H.M.T., 2014. Behavioral effects of endogenous or exogenous estradiol and progesterone on cocaine sensitization in female rats. *Braz. J. Med. Biol. Res.* 47 (6), 505–514.

- Tatara, M.R., Gołynski, M., Radzki, R.P., Bienko, M., Krupski, W., 2017. Effects of long-term oral administration of methimazole on femur and tibia properties in male Wistar rats. *Biomed. Pharmacother.* 94, 124–128.
- Uccelli, A., Moretta, L., Pistoia, V., 2008. Mesenchymal stem cells in health and disease. *Nat. Rev. Immunol.* 8, 726–736.
- Wang, F., Jiang, Y., Huang, X., Liu, Q., Zhang, Y., Luo, W., Zhang, F., Zhou, P., Lin, J., Zhang, H., 2017. Pro-inflammatory cytokine TNF- α attenuates BMP9-induced osteo/odontoblastic differentiation of the stem cells of dental apical papilla (SCAPs). *Cell. Physiol. Biochem.* 41, 1725–1735.
- Wang, Y., Zhou, L., Li, C., Xie, H., Lu, Y., Wu, Y., Liu, H., 2015. Bone marrow-derived cells homing for self-repair of periodontal tissues: a histological characterization and expression analysis. *Int. J. Clin. Exp. Pathol.* 8 (10), 12379–12389.
- White, J.P.M., Cibelli, M., Urban, L., Nilius, B., McGeown, J.G., Nagy, I., 2016. TRPV4: molecular conductor of a diverse orchestra. *Physiol. Rev.* 96, 911–973.
- Yang, J., Ye, L., Hui, T., Yang, D., Huang, D., Zhou, X., Mao, J.J., Wang, C., 2015. Bone morphogenetic protein 2-induced human dental pulp cell differentiation involves p38 mitogen-activated protein kinase-activated canonical WNT pathway. *Int. J. Oral Sci.* 7, 95–102.
- Yaoita, Y., 2019. Tail resorption during metamorphosis in *Xenopus* tadpoles. *Front. Endocrinol.* 10 (143), 1–11.
- Zeichner-David, M., 2006. Regeneration of periodontal tissues: cementogenesis revisited. *Periodontology 2000* (41), 196–217.
- Zheng, J., Kong, Y., Li, Y., Zhang, W., 2019. MagT1 regulated the odontogenic differentiation of BMMSCs induced by TGC-CM via ERK signaling pathway. *Stem Cell Res. Ther.* 10 (48), 1–13.