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Influence of Lithium On Micro-Hardness of Dental Tissues

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ABSTRACT

Lithium in pregnancy is related to the risk of preterm birth. The study is related to the influence of Lithium in which female rabbits were involved as an experimental model, the drug was given during pregnancy which might instigate the defects of teeth in their developing offspring which were utilized as a sample to evaluate the micro-hardness of dental tissues i.e. enamel and dentine. **Objective:** To evaluate the influence of lithium on the micro-hardness of dental tissues. **Methods:** The subjects were divided into two sets i.e. investigational and regulator, containing seven subjects per cluster (n=7), the sum of 168 samples. Micro-hardness was estimated on two tissues of teeth. Vickers Hardness gradation was tested by taking three indentations on each sample for enamel and dentine, distinctly with 50 gm of weight for 15 sec. **Results:** The statistical analysis was done by applying Student's t-tests using SPSS version 25. A noteworthy decline occurred in maxillary incisors, premolars, and molars, with p-values of 0.001, 0.012, and 0.003, separately. Comparatively, mandibular teeth, affected the incisors and molars, with p-values 0.003 and 0.011. Hardness affected the dentine of maxillary incisors, premolars, and molars, with p-values of 0.006, 0.005, and 0.004 individually. Micro-hardness in dentine was lowered in all the maxillary and mandibular teeth except mandibular molars. **Conclusions:** It was concluded that the tested values indicated the drug's effect on maxillary and mandibular teeth by reduced standards. The clinicians should therefore be careful prescribing the medication, particularly during pregnancy, eluding the unfortunate consequence of neonate's teeth.

INTRODUCTION

Publications up to 2023 were reviewed through a systematic search using electronic databases discovering the micro-hardness of enamel and dentine affected by Lithium. Prior reports revealed that Lithium escalation could be supportive in managing patients suffering from major depressive disorder (MDD) who do not or have a limited reaction to antidepressant usage [1]. Lithium in pregnancy is related to the risk of preterm birth. The risks and benefits of management would be consumed to monitor the policymaking, as recommended by Hastie *et al.*, [2]. In another study, it was noticed that individuals with Type1 diabetes mellitus (T1DM) destructively triggered enamel and dentine micro-hardness [3].Thermal management can also deproteinize humanoid dentine devoid of effects on the mechanical aspect, while chemical deproteinization alters the mechanical properties and crystallography of dentine [4]. According to former studies on re-mineralization, all mediators utilized in the study of early carious lesions, the Fluoride polish showed the maximum micro-hardness with minimum enamel solubility (ES) related to re-mineralizing mediators [5].Ghelejkhani *et al.*, reported that Fluoride varnish amplified the enamel micro-hardness. Laser therapy before the application of re-mineralizing mediators did not improve the enamel fight to demineralization [6]. Zhang *et al.*, fictitious lithium and cobalt co-doped mesoporous bioactive glass nanoparticles (Li-Co-MBGNs) using a reformed sol-gel technique.The antibacterial action compared to Staphylococcus aureus and Escherichia coli, indicates the usage in bone tissue production.General, outcomes indicated the viability of the kind in bone tissue production [7]. An in vitro study by Borges *et al.*, in which they assessed the effect of pressed lithium disilicate, through Knoop micro-hardness (KHN) of a light-cured resin cement at two

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complexities.The increasing ceramic thicknesses condensed the micro-hardness of resin cement. Augmented gravity in resin cement exhibited abridged micro-hardness in entirely all considered individuals. Improved ceramic opacity decreased the KHN of resin cement at individual zeniths for altogether ceramics [8]. Kiełczykowska *et al.*, evaluated the impact of selenium on lithium content, and homeostasis of microelements i.e. iron, zinc, copper, and manganese in the kidney and liver of rats experiencing lithium disclosure.The results of selenium as an adjuvant to lithium therapy, only one dose of Selenium seemed necessary to clarify its influence on elementary microelements and lithium addition in organs for the period of lithium exposure [9]. The effect on Dentine revealed the consequence of nanoparticle-built intra-canal pharmaceuticals on the root dentine micro-hardness test in two sets, Calcium hydroxide in addition to a regulator cluster. Vickers hardness standards remained predictable. Graphene oxide-silver nanoparticles built intra-canal treatment presented minimum consequence on micro-hardness of root dentine equated for Calcium hydroxide [10]. The survey of the literature revealed an altered effect on the micro-hardness by the use of Lithium regarding the restorations of teeth, and the influence on different body organs, whereas in the current experimental study, the consequence of Lithium was explored on the teeth of offspring by giving the drug systematically during pregnancy to the female rabbits. This type of study has not been reported showing the effect of drugs on teeth during their developmental stages.

Exposure to medications such as lithium during pregnancy may adversely affect the development of dental tissues in offspring, particularly enamel and dentine micro-hardness, which are critical indicators of tooth strength and integrity. Although previous research has explored lithium's systemic effects and its role in dental materials, there is a paucity of experimental evidence assessing its direct impact on developing teeth during the prenatal stage. Moreover, limited animal-based studies have quantified these structural changes using standardized micro-hardness testing. Therefore, this study aims to evaluate the effect of maternal lithium exposure on the micro-hardness of enamel and dentine in offspring, using a controlled experimental rabbit model.

METHODS

The quasi-experimental study design was planned, utilizing female rabbits as an experimental model. The study was conducted from January 2024- July 2024. Female rabbits were chosen for their reproductive physiology and hormonal relevance to lead toxicity and liver health studies. Subjects (1.5–2.0 kg) were divided into control and treated groups (n=7 each). The acceptable sample size (E=10–20) was calculated by subsequent formula [11]: E=Total number of animals – Total number of groups. The study was conducted at Baqai Medical University and the female rabbits were taken from the animal house of the University. The spell of the study was about two years. Female rabbits of 1.5 to 2.0 kg were designated for trial. Healthy offspring at the age of three months were used. Male rabbits, animals less than 1.5 to 2.0 kg, Subjects more than three months of age, and animals stated as unhealthy by the veterinary surgeon with any injuries, rashes, or edema were excluded from the study. Unhealthy subjects were excluded based on veterinary examination and/or laboratory tests, ensuring only healthy individuals were included. Lithium Carbonate is available under the professional name (Neurolith® SR. 400 mg) [12]. The required dose of the selected medication was prepared considering the total mass of the subject, meticulously according to Clark's rule [13]. Dose=Adult dose × (Weight Kg / 70). Preparation of the medication was done by dissolving one tablet in 10 ml of distilled water, and 3 ml (80 mg/kg) of the medication was given orally, two times per week to female rabbits of experimental subjects throughout the phase of pregnancy till the delivery of their offspring. These offspring were sacrificed at the age of three months for the investigational purpose of acquiring their maxilla and mandible. Extraction of teeth was executed including all three classes of teeth i.e. central Incisor, first Premolar, and the first Molar originating from the upper and lower jaws. Twelve samples (teeth) were engaged from the subjects of the study and regular groups, the total number of samples for the experimental purpose was 168. This benchmark could extract sufficient evidence to depict the conclusion. The methodology of sample preparation for testing micro-hardness was conducted according to the same procedure described in one of our published articles [14]. Samples preparation was accomplished by "Cold mounting" using Epoxy mount resin (Diglycidyl Ether Resin) and epoxy mount hardener (N-Amino-ethyl-piperazine). Resin and the hardener were placed inside the moulds (in grams) of 10:3 which were hardened at room temperature. Teeth were set in vertical positions and placed in a mould for informal admittance to enamel and dentine. The dimension of the mould was related to the diameter/height i.e. 30/15 mm, according to the size of the teeth. The transparent mounting material enabled for easy identification and location of the dental tissues. Grinding of the specimens was done after hardening of the samples before the polishing procedure. It was steered by waterproof emery papers having Si-C (Silicon Carbide) bits of 180, 220, 320, 400, 600, 800, and 1000 grit dimensions, with continuous water irrigation. Grinding machine, (Model No. Maopao 260 E). The polishing of samples was conducted on the polishing appliance (Model No. DUO 12 Benetec). The polishing spinning wheels were shielded with a fabric saturated by a reasonable abrasive material, Alumina (Aluminum Oxide: Al_2O_3) suspension in H20 progressively with 0-500 rpm. A Vickers hardness testing machine (Model

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No.402 MVD), was used to determine the hardness of the tooth structure. The diamond-shaped square-based pyramid of 136o was enforced on the refined surface of the sample, positioned on the appliance podium underneath the precise load of 50 gm with a Dwell time of 15 secs for individual samples. Three indentations for the enamel and dentine were taken. The magnitudes of the indentation were recorded in the automatic machine for scheming the micro-hardness of the dental tissues. The following formula was applied to calculate the micro-hardness [15]. HV=1.854 L / d2. Whereas: L=Denotes the applied load on the indenter in kg, D= mean diagonal of indentation, in mm and H=Vickers micro-hardness degree (kg/mm2). The images were perceived using an optical microscope (Model No. MMD-GX 51 Olympus), to focus on a high-quality expanded image. Pictures of the trial models were reserved at 100 X magnification. The data were analyzed on SPSS version 25. Descriptive analysis was accomplished by computing mean and standard deviation while inferential analysis was prepared by relating an independent t-test after examination of the data for normality. By using the Shapiro-Wilk test, the significance level was kept at 0.05.

RESULTS

The results indicated variations in the toughness (HV) values of both tissues i.e. enamel and dentine. A noteworthy reduction in the micro-hardness was perceived in maxillary incisors, premolars, and molars, showing the p-values as 0.001, 0.012, and 0.003, correspondingly, while in mandibular teeth, incisors, and molars were affected, with the p-values 0.003 and 0.011, mandibular premolars were least affected. Results of dentine showed a significant reduction of hardness in maxillary incisors, premolars, and molars, having the p-values 0.006,0.005, and 0.004. However minimal change in dentine micro-hardness of mandibular teeth was observed. The particulars of micro-hardness were summarized with statistical analysis to conclude. The values of Vickers hardness (HV) are represented through mean and standard deviations of the dental tissues i.e. Enamel and dentine comprising of control in addition to the experimental group of both maxillary and mandibular teeth expressed in table 1.

Table 1: Micro-Hardness of Dental Tissues Treated with Lithium

Samples (Teeth)	Micro-hardness- Unit H.V Mean ± SD (n=7)					
	Enamel			Dentine		
	Control	Treated	p-value	Control	Treated	p-value
Maxillary Incisor	246.27 ± 17.09	187.80 ± 34.97	0.001	52.174 ± 6.16	52.17 ± 6.16	0.006
Maxillary First Premolar	236.40 ± 43.97	181.36 ± 22.72	0.012	34.960 ± 3.12	34.96 ± 3.12	0.005
Maxillary first Molar	266.30 ± 33.92	198.27 ± 12.43	0.003	31.419 ± 2.48	31.42 ± 2.48	0.004
Mandibular Incisor	264.84 ± 21.03	208.27 ± 9.62	0.003	49.617 ± 6.19	49.62 ± 6.19	0.058
Mandibular First Premolar	239.82 ± 42.02	225.10 ± 18.90	0.414	36.507 ± 4.21	36.51 ± 4.21	0.044
Mandibular First Molar	250.18 ± 23.95	199.46 ± 37.96	0.011	41.220 ± 3.03	41.22 ± 3.03	0.570

Images of indentation at three locations on Enamel and dentine on the prepared samples are shown in Figure 1.

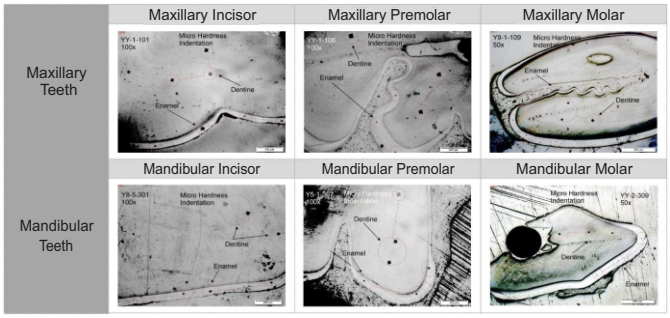

Figure 1: Photographic Representation of Vickers Hardness On Enamel and Dentine Showing Indentation On Three Points

The contrast of micro-hardness in the tissue of teeth i.e. enamel in the group of maxillary arches is presented in figure 2.

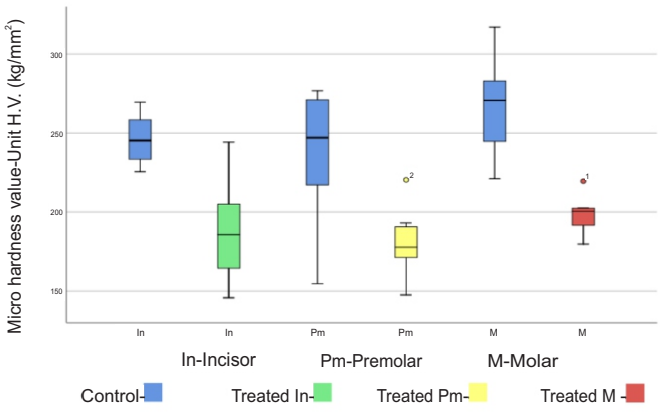

Figure 2: Micro-hardness of Enamel in Maxillary Teeth

Those of mandibular teeth are within reach in figure 3.

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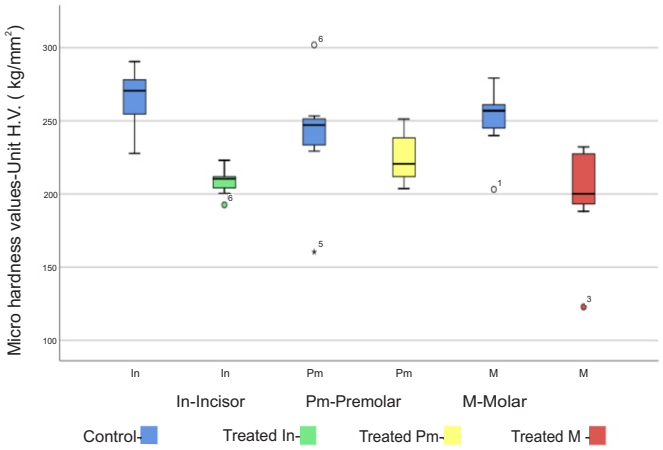

Figure 3: Micro-Hardness of Enamel in Mandibular Teeth

The comparable data of hardness values of dentine in normal and trial groups was analyzed. Micro-hardness of the dentine of maxillary teeth is represented in figure 4.

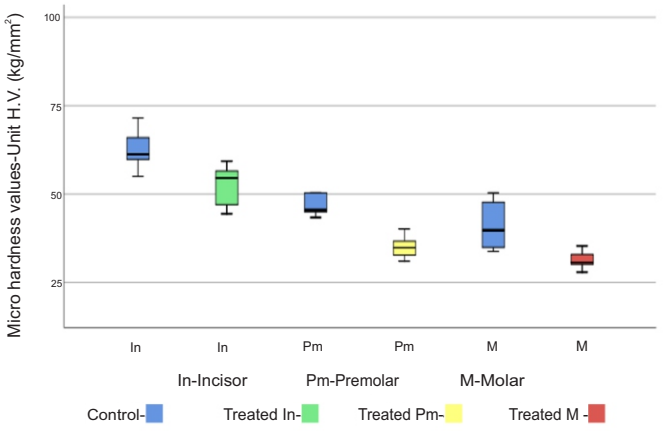

Figure 4: Micro-Hardness of Dentine in Maxillary Teeth

The dentine in mandibular teeth is interpreted in figure 5.

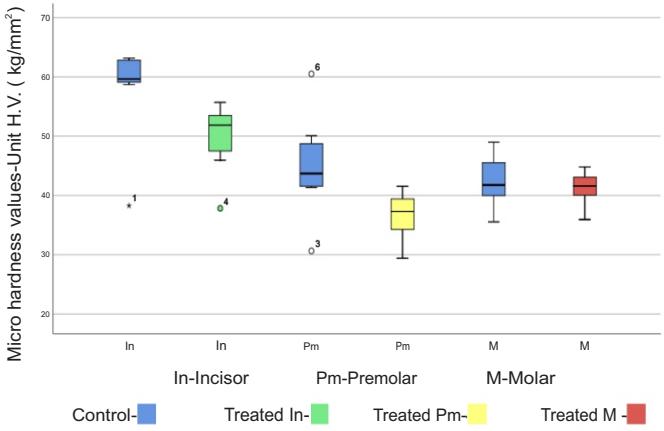

Figure 5: Micro-Hardness of Dentine in Mandibular Teeth

DISCUSSION

Little information is available on the prevalence of enamel defects in the developing stage affected by the administration of medication. Adversative drug responses could implicate all organs of the body. Assessment of micro-hardness in enamel and dentine is important related to dental hard tissue which is another degree for the 'healthiness' of these tissues. Micro-hardness measurement using the VHN test is an accepted technique. The Vickers Pyramid Number (HV) or Diamond Pyramid Hardness (DPH) has been documented as the Unit of the hardness [16].The hardness of the teeth was restrained using a Vickers diamond indenter in a typical micro-hardness test by Chun *et al.* The hardness rate of Enamel samples remained around (HV= 274.8 ± 18.1) which was about 4.2 times greater as compared to dentine (HV= 65.6 ± 3.9).It was reported that Enamel conceived greater resistance appropriate for grinding the foods, and dentine had upper strength resistance.The dissimilar parts of dental tissues may give diverse conformations along with interior features, as discovered by scanning electron micrographs [17]. In the contemporary study, mean micro-hardness standards of enamel in control and treated subjects were revealed as (236.40 ± 43.97 to 266.30 ± 33.92) and (181.36 ± 22.72 to 225.10 ± 18.90) respectively, regarding the dentine-tested hardness value of control (31.419 ± 2.48 to 52.174 ± 6.16) and treated (31.41 ± 2.48 to 52.174 ± 6.16) the results appeared in line with the reported values. Gaining additional understanding of the effects of these medications on the teeth of small mammals like rabbits. In the current study, a range of doses was administered to rabbits, meant to determine the effects of a selected drug on the developing teeth of their offspring, the results of such tests have been reported in the existing study. The results revealed that micro-hardness was significantly reduced in the enamel of all the maxillary teeth. Micro-hardness values showed a noteworthy reduction in dentine except for the mandibular molars.According to Chuenarrom *et al.*, the alteration of the denting period did not affect the micro-hardness values of dental tissues. The Knoop hardness numbers (KHN) standards of Enamel and Vickers hardness numbers (VHN) of Dentine were provoked by differences in the applied loads.Hence, the tooth firmness figure for diverse loads may not be suitable for evaluation with the results.The study was conducted by applying different test loads [18].Relating to the present study, a variation in the micro-hardness after the use of a drug was channeled by applying a particular weight of 50 gm with Dwell time of 15 sec.This parameter was kept uniform for all the samples.There is a dearth of published data regarding the consequences of Lithium on evolving teeth. However, the consequences of the drug's effect on developing teeth are presented in the contemporary study. Moreover, there is scarce evidence about neonatal imperfections following in-utero exposure of Lithium.The practice of the Vickers hardness tester for assessing the standards of enamel micro-hardness e.g. diamond burs

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adversely affected enamel micro-hardness and meaningfully reduced it.Standards of healthy enamel micro-hardness were attained by 40 and 60 µm instead of 90 µm abrasive strips. Using 15 µm abrasive strips and Sof Lex abrasive discs, the micro-hardness standards acquired remained greater, related to those documented for healthy enamel [19]. Influence on alveolar bone reported by Wadke *et al.*, that lithium might improve alveolar bone development for the period of orthodontic retention, which might influence the orthodontic management period in patients getting lithium, and orthodontic tooth movement (OTM) [20]. Micro-hardness of root dentine indicated reduction by a gold standard intra-canal medicament i.e. Calcium hydroxide (CH). A natural extract, propolis, acted better to CH in eliminating endodontic microbes, A Vickers hardness indentation appliance with a load of 200 g and dwell period of 15 s at 24 h, 3, and 7 days were cast off for micro-hardness analysis. After 7 days, it was established that the highest micro-hardness assessment was (64.43 ± 1.69), while CH was revealed to be the lowermost value as (48.46 ± 1.60). The root dentine micro-hardness improved with the application of propolis, while it reduced dentine sections after the use of CH [21]. Published literature provided information regarding the micro-hardness of human teeth and the use of Lithium for restoration procedures in dental practice. Micro-hardness of dentine was also assessed by the use of intra-canal medicament. On the other hand, the present study was conducted on a different constraint to assess the micro-hardness of enamel and dentine on developing teeth influenced by a medication administered to the mother during pregnancy, such kind of study has not been described in the published literature.

However, the present study is limited by its small sample size, use of a single animal model, and controlled laboratory conditions, which may not fully replicate human biological responses, thereby restricting generalizability. Future studies should include larger sample sizes and multiple animal models to enhance external validity and explore dose-response relationships of lithium exposure. Longitudinal and human-based studies are also recommended to assess clinical relevance and long-term dental outcomes. Additionally, investigating preventive or protective interventions could provide clinical value.

CONCLUSIONS

It was concluded that a better understanding of the mechanical properties of enamel and dentine may facilitate the practitioners to relate and apply it in clinical practice. It was thus comprehensible that Lithium could lead to developmental defects of dental tissues, therefore it must be considered obligatory to take the medication by doctor's recommendation during pregnancy to escape the risk of developing defects in newborns.

Authors' Contribution

Conceptualization: SN
Methodology: SN
Formal analysis: SN
Writing and Drafting: SN, AM
Review and Editing: SN, AM

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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