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ORIGINAL ARTICLE

Observation of Dental Structures Under the Influence of Acids: An In-Vitro Forensic Study

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ABSTRACT

Context: Forensic odontology plays a vital role in human identification, particularly when conventional methods are hindered by decomposition or trauma. Understanding the effects of various environmental factors on teeth is crucial for effective forensic analysis.

Aims: The study aim to evaluate the effects of three different acids - 37% Hydrochloric Acid (HCl), 65% Nitric Acid (HNO_3), and 98% Formic Acid (HCOOH) - on extracted permanent mandibular first molars, focusing on non-carious, carious, and Class I composite filled teeth.

Methods and Material: A total of 45 extracted permanent mandibular first molars were subjected to acid solutions each of 30 ml for different durations ranging from 15 minutes to 24 hours. The teeth were categorized into non-carious, carious, and Class I composite-filled groups. Morphological, microscopic, and radiographic evaluations were conducted at various time intervals to assess the effects of acid exposure on the dental tissues and restorations.

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Results: The study found that acid exposure led to significant changes in the morphology, microscopic structure, and radiographic appearance of both dental tissues and restorations. Variations in the rate of degradation were observed depending on the type of acid and the condition of the teeth.

Conclusions: The results offer valuable insights into the effects of acid exposure on teeth, particularly in forensic odontology. Understanding these effects is critical for evaluating the durability of dental structures and restorations in forensic identification processes, where teeth are frequently used as a primary means of identification.

KEYWORDS

• Forensic Odontology • Human Identification • Acid Exposure • Morphological Changes • Microscopic Evaluation

Key Messages: The present study highlights that exposure of teeth to various acids significantly affects the morphology, microscopic structure, and radiographic appearance of both natural tooth and restorations. Understanding these effects is crucial for forensic odontology, as it helps assess the durability of teeth and restorations in human identification processes, especially when traditional methods are challenged by decomposition or trauma.

INTRODUCTION

Forensic odontology, specialized branch of forensic medicine, involves the analysis and interpretation of dental structures and materials to aid legal investigations.[1] Forensic odontology has emerged as one of the most intriguing branches of forensic sciences in recent years.[1] It has become increasingly vital in medico-legal matters and in identifying deceased individuals. In the modern era, with its unique challenges, human identification remains a complex issue. Dental identification has gained prominence due to its reliability and resilience compared to other human tissues, making teeth significant in forensic investigations.[2-4]

Over the years, it has developed into a crucial component of forensic science, particularly in cases involving human identification.[5] Given the resilience of dental tissues, especially under extreme conditions, teeth often serve as a primary means of identifying individuals when other anatomical features are unrecognizable. Their high mineral content approximately 96% in enamel compared to 70% in bone enables them to withstand decomposition, high temperatures exceeding 1000°F, and physical trauma. Posterior teeth are better preserved in catastrophic events due to protection from surrounding soft tissues like the tongue, facial muscles, and fat.[1]

The materials placed in the oral cavity, including crowns, fillings, bridges, dentures, and implants, are engineered to endure mechanical stress, contributing to their survival in forensic scenarios. This durability enhances the evidentiary value of dental components, making them indispensable tools in modern forensic investigations, especially when traditional identification methods fail.[6]

SUBJECTS AND METHODS

Sample selection: After obtaining Institutional Ethical Clearance a total of 45 extracted permanent mandibular first molars were used in the current study. The sample included three categories of teeth: non-carious, carious, and Class I composite filled, all of which were extracted as they were periodontally compromised.

Data collection: The extracted teeth were collected from the Department of Oral and Maxillofacial Surgery Department at Manubhai Patel Dental College.

Teeth were initially rinsed with tap water, brushed to remove residual soft tissue, and disinfected in 10% formalin. Calculus deposits were cleared using an ultrasonic scaler prior to acid exposure.

The specimens were divided into three experimental groups, as follows:

1. **37% Hydrochloric Acid (HCl) group**: 15 extracted tooth specimens
2. **65% Nitric Acid (HNO_3) group**: 15 extracted tooth specimens

3. **98% Formic Acid (HCO_2H) group**: 15 extracted tooth specimens

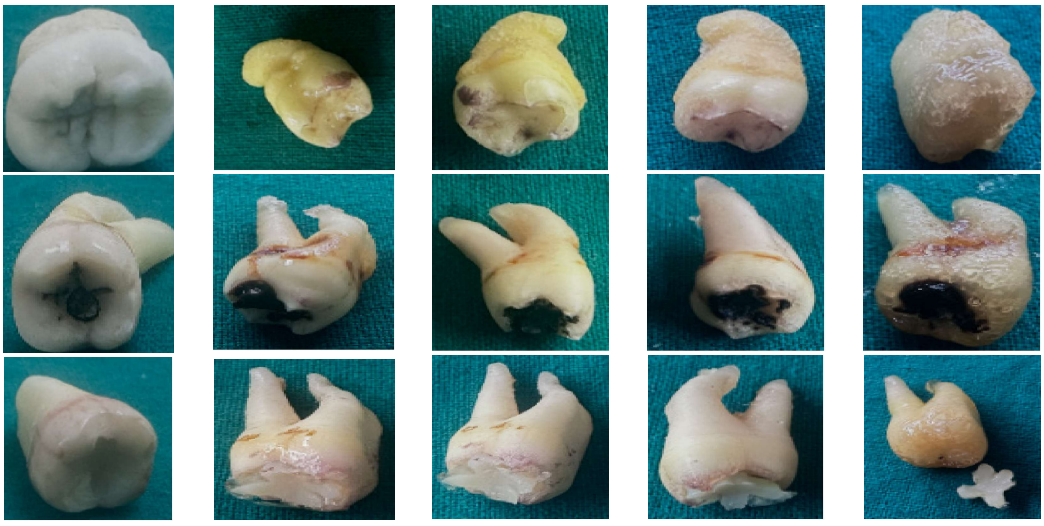
Each group of 15 specimens were subjected to the respective acid solution each of 30 ml and observed for a period of 24 hours. At various intervals (15 min, 30 min, 1 h, 3 h, 5 h, and 24 h), the samples were removed from the solutions, and morphological, microscopic, and radiographic images were captured. The specimens were then returned to the acid solutions for further exposure. The following changes were monitored and recorded: effervescence, color changes, transparency alterations, disintegration, complete dissolution, and precipitation.

RESULTS

Sequential morphologic, microscopic and radiographic changes along with duration in normal teeth, carious teeth and composite filled teeth after the immersion of tooth specimens in 35% Hydrochloric Acid shown in *Table 1* and *Figure 1*.

Table 1: Morphological Changes in Teeth Following Immersion in 30 mL of 35% HCl Solution

Acid	Duration of Exposure	Morphologic Changes	Microscopic Changes	Radiographic Changes
Hydrochloric acid (HCL)	30 mins-2 hours	Normal teeth-Effervescence observed in acid solution. After 1 hour, erosion with pointed elevations appears on the occlusal surfaces. Demineralisation of the carious portion seen. The composite restoration remains unaffected	The surface of the teeth appeared glossy with few brown stains. Occlusal surface shows pinpoint elevations due to erosion	Reduction in overall size was seen at 30 mins. At 1 hour Enamel degradation was evident, with the curvature becoming uniformly smooth
	2-4 hours	By 2 hours, the teeth become translucent at the occlusal surface. The translucency progressively spreads around the teeth in the following hours. Brown stains were seen on the teeth.	The occlusal surfaces of the teeth showed translucency with pinpoint elevation and brown stains.	The root portion exhibited the presence of fine fracture lines.
	4-8 hours	Concentric circles appeared on the root at 4 hrs. After 8 hrs, teeth were gelatinous and eventually started floating in the acid solution. Carious-Composite	The teeth show concentric colours and translucency at the root apex.	No relevant changes were noticed.
	8-24 hours	Teeth sized were reduced overall, but they were not completely dissolved after 24 hours.	Teeth appeared completely translucent with brown stains. Composite restoration is dislodged from the teeth	The teeth size was remarkably reduced, with radiolucent margin covering the teeth.

Time	30 mins	1 hour	3 hours	5 hours	24 hours
Morphological Changes					

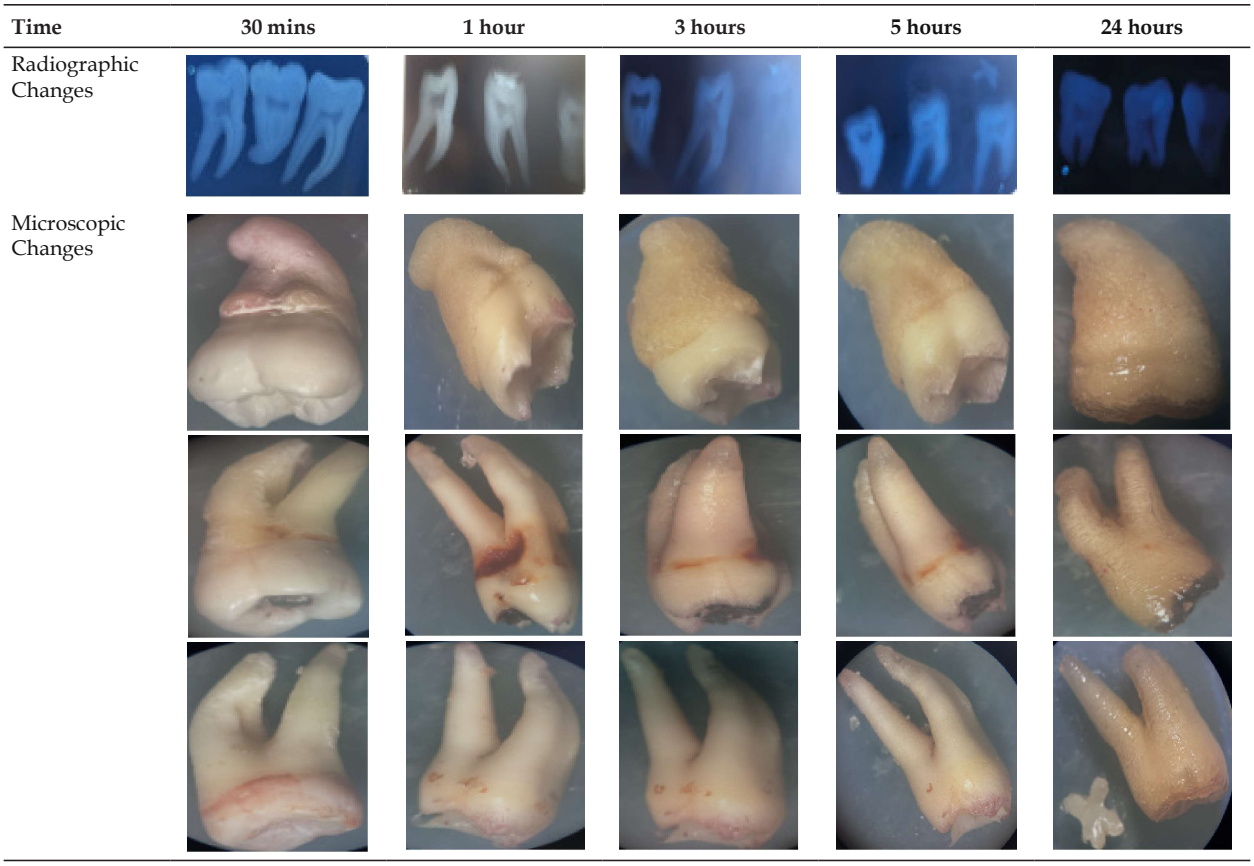


Figure 1: Progressive Morphologic, Microscopic, and Radiographic Alterations in Teeth Exposed to 30 mL of 35% HCl

Sequential morphologic, microscopic and radiographic changes along with duration in normal teeth, carious teeth and composite filled teeth after the immersion of specimens in 63% Nitric Acid shown in Table 2 and *Figure 2*.

Table 2: Morphological Changes in Teeth Following Immersion in 30 ml- 69% conc. HNO_3 (Nitric Acid)

Acid	Duration of Exposure	Morphologic Changes	Microscopic Changes	Radiographic Changes
Nitric Acid (HNO_3)	30 mins-2 hours	Effervescence observed in acid solution. Demineralisation of enamel. Yellow colour residue seen all over the teeth Carious- white deposition seen on the carious surface Composite- No effect seen with the composite restoration	Brownish discolouration seen on the occlusal surface with pinpoint elevations. Mineralisation of the carious portion. No effects noticed with the composite restoration	No fracture lines observed; enamel surface shows deterioration; tooth surface outline appears smooth
	2-4 hours	Teeth size reduced overall. Teeth show gel like consistency and the surface texture of teeth becomes sticky in nature	Teeth appeared smaller and yellow discoloration seen on crown and root surfaces. Carious portion appeared white and composite restoration in intact	Enamel surface shows deterioration; multiple fracture lines observed in the root portion
	4-8 hours	Gelatinous layer formed over the teeth	Translucent layer formed covering the teeth with concentric circles like crack seen all over the crown and root.	A noticeable reduction in root thickness was observed, along with complete structural deterioration of the crown

8-24 hours	Disintegration of teeth started, but teeth were not completely dissolved after 24 hours. Teeth exhibited complete loss of morphological structure.	Overall teeth size reduced. Concentric circle become more evident on the teeth surfaces. Total demineralisation of the carious portion. Composite restoration dislodges from tooth structure	Teeth lost their complete morphology	

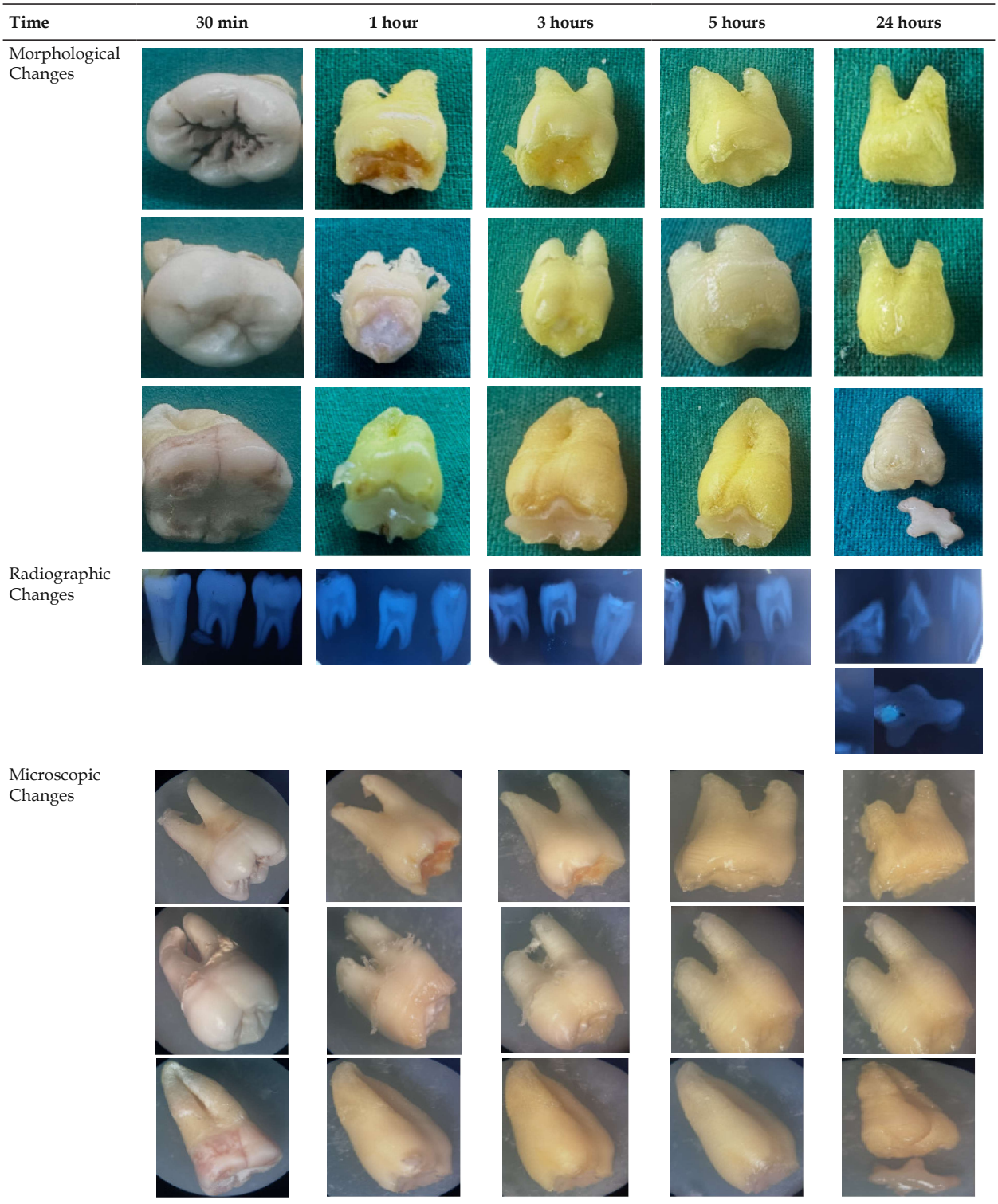
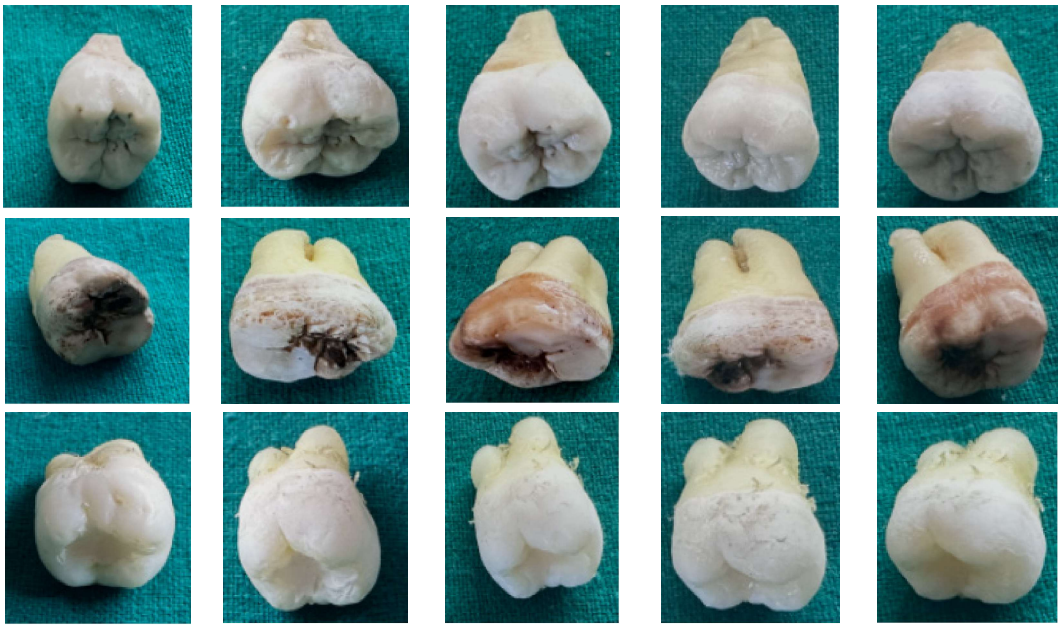


Figure 2: Progressive morphologic, microscopic, and radiographic alterations in teeth exposed to 30 ml- 69% conc. HNO_3

Sequential morphologic, microscopic and radiographic changes along with duration in normal teeth, carious teeth and composite filled teeth after the immersion of specimens in 98% Formic Acid show in *Table 3* and *Figure 3*.

Table 3: Morphological Changes in Teeth Following Immersion in 30 ml - 98% conc. HCOOH (Formic Acid)

Acid	Duration of Exposure	Morphologic Changes	Microscopic Changes	Radiographic Changes
Formic Acid (HCOOH)	30 mins - 2 hours	No significant morphological changes were observed. No changes observed with the carious portion and composite restoration remained intact	No significant changes. Teeth size appears normal	No significant changes observed. Teeth appears normal
	2-4 hours	No efferve scence was seen in the acid. All teeth appeared normal with no to minimal morphological changes. No demineralization is seen with the carious portion and composite restoration remains intact and unaffected.	No significant changes. Teeth size appears normal	No significant changes observed. Teeth appears normal
	4-8 hours	White deposits were seen on the crown, root surfaces remained unaffected. No effects observed for the carious portion and the composite restoration	Chalky white patches seen on the teeth surfaces. No changes seen with carious portion and composite restoration	No significant changes observed. Teeth appears normal
	8-24 hours	No changes in overall size of the teeth. Chalky white appearance seen covering the crown of the teeth and few spots on the root surface. Carious portion and composite is also covered with white deposits.	Loss of gloss and surface appears matt with chalky white appearance. Composite restoration remains intact with the teeth surface. Carious portion remains unaffected	Surface irregularities seen with no changes in the size of the teeth. Enamel appears more radiopaque. No change observed in the carious portion. Composite remained intact within the teeth

Time	30 min	1 hour	3 hours	5 hours	24 hours
Morphological Changes					

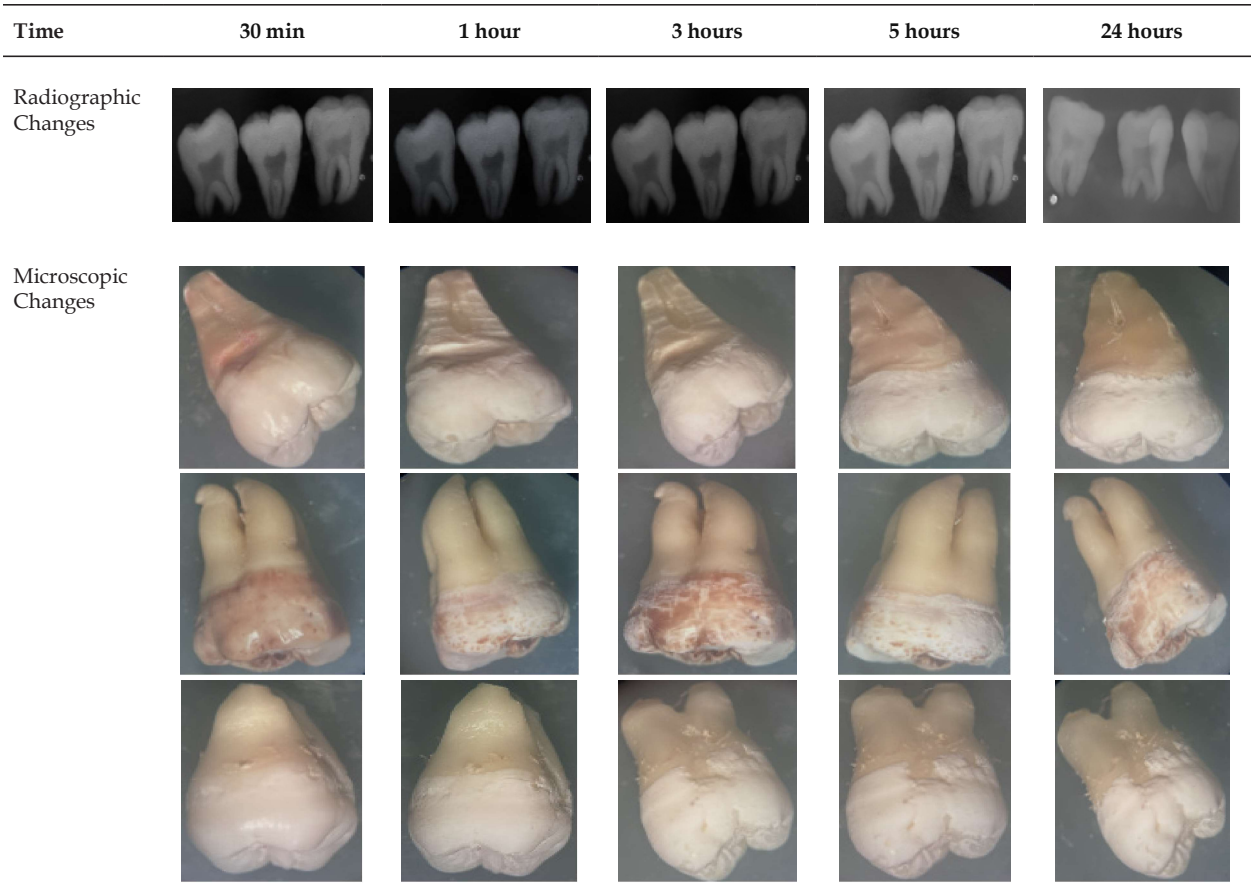


Figure 3: Progressive Morphologic, Microscopic, and Radiographic alterations in teeth exposed to 30 ml- 98% conc. HCOOH

DISCUSSION

Acids are commonly used in laboratory procedures and, unfortunately, in criminal activities like erasing personal identification. In such cases, dental tissues play a crucial role in identifying age and gender. Human teeth are durable and provide valuable forensic evidence, helping distinguish human from animal teeth, identify tooth type, and detect restorations.[9] Dental professionals are responsible for maintaining accurate records, which assist in identifying individuals and uncovering malpractice or fraud.[8]

Recent shifts in dental practices emphasize restorative treatments over extractions, aiming to preserve tooth structure. Given the rising incidence of dental caries, understanding how acids affect tooth structure and restorative materials is increasingly important.[8] Teeth, despite being the hardest body structures, can be damaged by acids, making restorations valuable for identifying individuals in acid-related crimes. This highlights the importance of dental knowledge in forensic investigations.[9]

In this study, the morphological, radiographic, and microscopic changes in teeth exposed to various acidic media were evaluated. The findings indicate that teeth do not exhibit uniform responses across all acid types.

Notably, exposure to 35% hydrochloric acid (HCl) and 69% nitric acid (HNO_3) resulted in complete morphological loss. However, in the HNO_3 medium, a yellow-colored deposit was observed, likely due to nitrate formation during the chemical reaction. In contrast, teeth exposed to formic acid (HCOOH) did not undergo dissolution; instead, a white precipitate formed, suggesting the creation of an insoluble salt. These observations clearly demonstrate the varying destructive potentials of different acids.[7]

Kadashett V et al. (2021)[9] reported that teeth in hydrochloric acid were completely dissolved after 18–20 hours, and in nitric acid, they dissolved after 16 hours. In the present study, while teeth exposed to hydrochloric and nitric

acids showed marked reduction in size after 24 hours but they were not completely dissolved, indicating that the dissolution process was less aggressive or slower than in the study by Kadashett *et al*.

Khushdeep *et al*. (2020)[7] found that the morphological and radiographical appearance of teeth persisted for up to 8 hours in hydrochloric acid, with no changes observed after 24 hours in 85% phosphoric acid. In line with these findings, the present study also observed that teeth exposed to hydrochloric acid maintained their morphological and radiographical appearance for 8 hours. Additionally, no morphologic or radiographic changes were noted in teeth exposed to formic acid after 24 hours, further supporting the findings of Khushdeep *et al*. **Thanuja *et al*. (2018)**[8] noted that composite restorations in teeth exposed to hydrochloric acid were dislodged at 20 hours, and those in nitric acid were dislodged at 24 hours. Similarly, in the present study, composite restorations were dislodged at 20 hours in hydrochloric acid and at 24 hours in nitric acid. However, composite restorations remained intact in formic acid even after 24 hours, consistent with the findings reported by Thanuja *et al*.

Raj *et al*. (2013)[10] noticed cracks ranging from mild to deep in the teeth within 15 to 30 minutes of acid exposure. Dissolution commenced within 1 hour, with complete structural breakdown occurring by 8 hours in both nitric acid (HNO_3) and hydrochloric acid (HCl). The present study, however, found that cracks appeared after 8 hours, and no dissolution was observed even after 24 hours of exposure to either hydrochloric or nitric acid, suggesting a slower progression of the dissolution process in the current study. **Jadhav *et al*. (2009)**[11] reported that teeth exposed to nitric acid displayed a yellow color within 15 minutes, followed by the disintegration of the apical third of the tooth evident at 4 hours, splitting of the teeth observed by 8 hours, and complete dissolution occurring by 15 hours. The present study observed a similar yellow color after 15 minutes when teeth were exposed to nitric acid, but the teeth were not completely dissolved even after 24 hours, indicating a slower or less severe dissolution process.

Cope and Dupras (2009)[12] reported that teeth immersed in hydrochloric acid developed a *"jelly-like"* appearance. However, in the present study, teeth exposed to hydrochloric acid exhibited outer translucency, indicating a differing morphological response compared to previous findings. **Mazza *et al*. (2005)**[13] observed that the morphological and radiographic characteristics of teeth remained intact even after 24 hours of exposure to formic acid, suggesting a less aggressive effect on dental structures when compared to sulfuric and phosphoric acids. In their study, the radiographic appearance of teeth exposed to sulfuric and phosphoric acids persisted up to 24 hours, with no significant changes observed until after 5 hours. In contrast, the present investigation demonstrated earlier onset of morphological and radiographic changes in response to these acids, highlighting a potentially more pronounced destructive effect under the tested conditions.

CONCLUSION

This study is unique in using three criteria morphological, radiological, and microscopic changes to evaluate the impact of different acids on dental structures, offering a comprehensive approach for forensic investigations. The results show that teeth immersed in formic acid exhibited minimal dimensional changes (less than 5 mm) after 24 hours, while those exposed to nitric acid (HNO_3) and hydrochloric acid (HCl) experienced significant reductions, making them unsuitable for further analysis. The durability of teeth highlights their potential as a valuable forensic identification tool. Morphological and radiographic changes at specific intervals of acid immersion can help identify the acid used and estimate exposure duration. However, final acid identification should be confirmed through biochemical tests. Additionally, acid effects on teeth depend on the type, concentration, duration of exposure, and factors such as tooth size and position.

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