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EVALUATION OF CYTOTOXIC MOLECULES RELEASED FROM ORTHODONTIC ADHESIVES - AN IN-VITRO STUDY

Orthodontics

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ABSTRACT

Background And Objectives: The toxicity of resin-based materials is due to residual monomers as well as to degradation products linked to activity of the salivary esterases. Elution of BPA may result from impurities left after synthesis of resin due to incomplete polymerization or degradation of the resin. **Aim**: The aim of the present study was to evaluate the release of cytotoxic molecules from orthodontic adhesives. **Methodology:** A total of 40 samples were taken for the study. Sample were divided into two groups: Transbond XT group (n=20) and other 3M Concise Ortho group (n=20). Both the samples were immersed in artificial saliva. After specific time intervals, samples were processed to extract cytotoxic molecules using ethanol, vortexing and centrifugation. The supernatant was evaporated and dissolved in methanol for HPLC analysis. HPLC was performed to evaluate BPA levels, with the analysis showing a progressive increase in BPA release over time for both adhesives. The study employed rigorous methods to standardize tooth specimens and analyze BPA levels accurately, highlighting the potential cytotoxic effects of orthodontic adhesives. **Results:** The study compared BPA release from chemical-cure (3M Concise Ortho) and light- cure (Transbond XT) composite adhesive. Both showed a progressive increase in BPA release over time. Statistical analysis revealed significant differences in BPA levels between the adhesives at all- time intervals, indicating the importance of adhesive selection in orthodontic treatments. **Conclusion:** Within the limitations of the study, due to small sample size, further research is warranted to investigate BPA release in vivo and to understand its potential long-term effects on oral health.

KEYWORDS

Resin-based materials, orthodontic adhesives, cytotoxicity, BPA release, artificial saliva, HPLC analysis.

INTRODUCTION:

The use of bonded brackets with adhesives has significantly advanced orthodontics, offering benefits over brackets welded to bands. However, research often focuses more on the physical characteristics of adhesives, like shear bond strength, rather than on biological compatibility.

Orthodontic resin-based adhesives contain two main components: an organic matrix based on functional di-methacrylates such as BISGMA, Urethane di-methacrylate, or triethyleneglycol di-methacrylate, and an inorganic filler component like silica, glass, and ceramic. During bonding, the vestibular mucosa may be exposed to the oral cavity's aqueous environment, leading to the dilution of monomers. This can cause health issues like allergic dermatitis, migraines, and behavioral abnormalities.

Monomers from adhesives may dissolve in saliva and form diluted solutions when in contact with the mucosa, particularly near the gingiva. This diluted fluid can be absorbed or inhaled, posing health risks.

Dental professionals must take safety measures to reduce monomer exposure, such as wearing safety gear, ensuring proper ventilation, and limiting exposure time. They play a crucial role in ensuring the safety and well-being of both patients and themselves. Incomplete polymerization of orthodontic adhesive due to oxygen inhibition can lead to the release of non-polymerized and potentially harmful materials. This incomplete polymerization affects the adhesive's mechanical properties and raises concerns about its biocompatibility and health effects.

Minimizing oxygen exposure during bonding and ensuring proper polymerization are crucial to reduce the release of non-polymerized components and maintain safety. Extensive research has investigated the toxicity of monomers like Bis GMA, BISGMA bis, TEGDMA hydroxyl ethyl methacrylate (HEMA), and urethane dimethacrylate (UDMA) found in polymerized resins. Studies show these monomers are cytotoxic and mutagenic, inducing oxidative stress within cells.

Degradation by-products of dental adhesives, like Bisphenol-A (BPA), can induce toxic effects similar to those of the original monomers. BPA, a precursor for Bisphenol A diglycidyl dimethacrylate (BIS-GMA) formulation, can mimic steroid hormones, leading to adverse biological effects.

While some studies have evaluated the cytotoxic effects of different adhesive components individually, considering the overall composition of adhesives and their degradation products is crucial.

High Performance Liquid Chromatography (HPLC)/mass spectrometry is a useful tool for detecting cytotoxic monomers in adhesives, allowing for reliable tracking of BPA release over time or across adhesive formulations.

The present study aims to determine the cytotoxicity of two orthodontic adhesives, Transbond XT and 3M Concise Ortho, in contact with artificial saliva. It also aims to evaluate the leaching of cytotoxic molecules, mainly BPA, from these adhesives at different time intervals. Understanding these effects is essential for ensuring the safety of orthodontic treatments.

MATERIALS & METHOD:

Source Of Data:

To assess the release of cytotoxic molecules from two different orthodontic adhesives - light cure and chemical cure, that are frequently used for bracket bonding procedures, an in-vitro experiment was carried out in the Department of Orthodontics and Dentofacial Orthopedics, ITS Dental College, Hospital and Research Centre, Greater Noida after taking approval from Institutional ethics committee.

Sample Size:

i. Sample size estimation was done by using GPower software (version 3.1). Sample size was estimated for "Means: Difference between two independent means" was chosen.
ii. A minimum total sample size of 40 materials (20 in each group) was found to be sufficient for an alpha of 0.05, power of 80%, 0.8 as effect size (to assess the significant difference between the groups).

Type Of Study /Study Design:

The design of the study is an in-vitro study.

Inclusion Criteria:
Healthy extracted premolars for Orthodontic purpose. (Fig. 1)

Exclusion Criteria:
- Carious or Fractured tooth. Root canal treated tooth.
- Tooth with restoration.
- Tooth with fluorosis.
- Any tooth other than premolar.

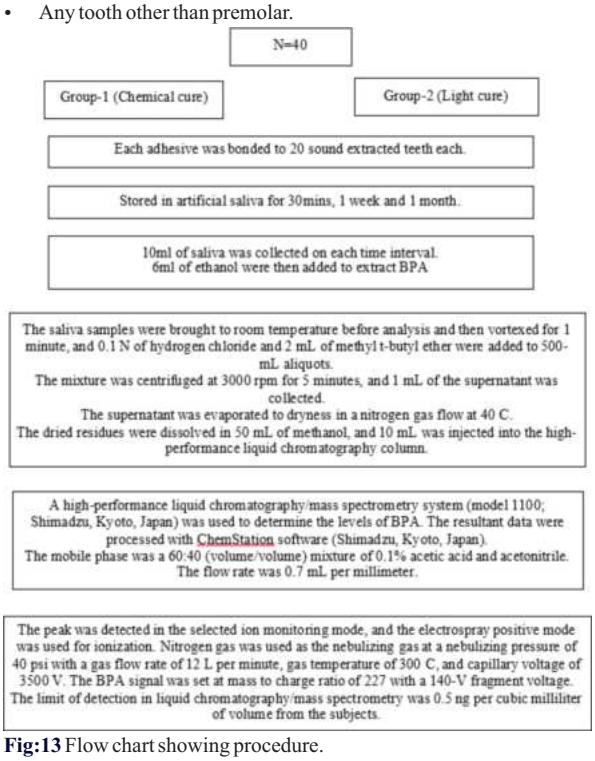

Fig:13 Flow chart showing procedure.

RESULT
The study was conducted to evaluate and compare the release of BPA from two different composite adhesives, namely light-cure and chemical-cure, when immersed in artificial saliva at intervals of 30 minutes (T1), one week (T2), and one month (T3) post-curing. High-performance liquid chromatography was employed to analyze BPA levels in all the samples.

Subsequently, the gathered data was tabulated and subjected to statistical analysis to discern any variations between the adhesives. This comprehensive approach aimed to provide insights into the potential differences in BPA release kinetics, contributing to a better understanding of the materials performance and potential implications for dental applications.

Intra-group comparison: For group 1: 3m concise ortho (chemical cure): BPA released in artificial salivary medium from Group 1 was found to be progressively increasing. At T1(30 minutes after curing) - 0.02691213607 (mean).

At T2 (24 hours after curing) 0.07356302649 (mean).0.07356302649 (mean) At T3 (1 month after curing) 0.09551932813 (mean). All these were found to be statistically significant as shown in Table 2

Table 2 BPA levels (mcg/dL) : Group 1 Concise ortho

Comparison of Group 1: 30 minutes, 1 week & 1 month.				
Group 1	Sample	Mean	Standard Deviation	P-Value
T1 (30 min)	20	0.02691213607	0.0009931571715063	0.000
T2(1 week)	20	0.07356302649	0.0009034870080501	0<0.0
T3(1 month)	20	0.0955193281	0.0009091790270385	5, S
There is a significant difference across the two groups				

Table 3 Comparison of BPA levels (mcg/dL) at T1 & T2 : Group 1 Concise ortho

Comparison of Group 1: 30 minutes versus 1 week				
Group 1	Sample	Mean	Standard Deviation	P-Value
T1 (30 min)	20	0.02691213607	0.0009931571715063	0.0000<
T2(1 week)	20	0.07356302649	0.0009034870080501	0.05, S
There is a significant difference across the two groups				

Table 4 Comparison of BPA levels (mcg/dL) at T2 & T3 : Group 1 Concise ortho.

Comparison of Group 1: 1 week versus 1 month				
Group 1	Sample	Mean	Standard Deviation	P-Value
T2 (1 week)	20	0.07356302649	0.0009034870080501	0.0000<
T3 (1 month)	20	0.09551932813	0.0009091790270385	0.05, S
There is a significant difference across the two groups				

Table 5 Comparison of BPA levels (mcg/dL) at T1 & T2 : Group 1 Concise ortho.

Comparison of Group 1: 30 minutes versus 1 month				
Group 1	Sample	Mean	Standard Deviation	P-Value
T1(30 min)	20	0.02691213607	0.00099315715063	0.0000<
T3(1 month)	20	0.09551932813	0.0009091790270385	0..05, S
There is a significant difference across the groups				

In the intra-group analysis of Group 1, comparisons revealed varying mean differences in BPA levels across different time intervals. Specifically, the differences were 0.04665089 mcg/dL between T1 and T2, 0.06866719 mcg/dL between T1 and T3, and 0.0219563 mcg/dL between T2 and T3 as shown in Tables 3,4 &5.

The most substantial disparity occurred between T1 and T3, amounting to 0.06866719 mcg/dL, while the smallest difference was observed between T2 and T3, at 0.0219563 mcg/dL.

Notably, these discrepancies were deemed statistically significant, underscoring the significance of temporal variations in BPA levels within the group.

For Group 2: Transbond XT (Light cure):
BPA released in artificial salivary medium from Group 2 was found to be progressively increasing. At T1(30 minutes after curing) - 0.02411914902 (mean).
At T2 (1 week after curing) -0.06725862487 (mean).
At T3 (1 month after curing) - 0.091334682738 (mean).

All these were found to be statistically significant as shown in Table 6

Table 6 Comparison of BPA levels (mcg/dL) at T1 & T2 : Group 2 Transbond XT.

Comparison of Group 2: 30 minutes, 1 week & 1 month.				
Group 2	Sample	Mean	Standard Deviation	P-Value
T1 (30 min)	20	0.02411914902	0.0006574946540779	0.000
T2(1 week)	20	0.06725862487	0.0011546157778869	0<0.0
T3 (1 month)	20	0.09134682738	0.0011930357769053	5, S
There is a significant difference across the groups				

Table 7 Comparison of BPA levels (mcg/dL) at T1 & T2 : Group 2 Transbond XT.

Comparison of Group 2: 30 minutes versus 1 week				
Group 2	Sample	Mean	Standard Deviation	P-Value
T1 (30 min)	20	0.02411914902	0.0006574946540779	0.0000<
T2(1 week)	20	0.06725862487	0.0011546157778869	0.05, S
There is a significant difference across the two groups				

Table 8 Comparison of BPA levels (mcg/dL) at T2 & T3 : Group 2 Transbond XT.

Comparison of Group 2: 1 week versus 1 month				
Group 2	Sample	Mean	Standard Deviation	P-Value
T2(1 week)	20	0.06725862487	0.0011546157778869	0.0000<
T3 (1 month)	20	0.09134682738	0.0011930357769053	0.05, S
There is a significant difference across the two groups				

Table 9 Comparison of BPA levels (mcg/dL) at T1 & T3 : Group 2 Transbond XT.

Comparison of Group 2: 30 minutes versus 1 month				
Group 2	Sample	Mean	Standard Deviation	P-Value

T1 (30 min)	20	0.02411914902	0.0006574946540779	0.0000<
T3 (1 month)	20	0.09134682738	0.0011930357769053	0..05, S
There is a significant difference across the two groups				

In the intra-group analysis of Group 2, comparisons also revealed varying mean differences in BPA levels across different time intervals. Specifically, the differences were 0.04313948 mcg/dL between T1 and T2, 0.06722768 mcg/dL between T1 and T3, and 0.02407606 mcg/dL between T2 and T3 as shown in Tables 7, 8 & 9.

The most substantial disparity occurred between T1 and T3, amounting to 0.06722768 mcg/dL, while the smallest difference was observed between T2 and T3, at 0.02407606 mcg/dL.

Notably, these discrepancies were deemed statistically significant, underscoring the significance of temporal variations in BPA levels within the group.

Inter-group Comparison:

By comparing the amount of BPA quantitatively among the samples collected at 30 minutes after curing and immersion in artificial saliva (T1), it was identified that Group 1 has comparatively more release and Group 2 has less release.

The inter group comparison at T1 is listed below (Table 10)

Table 10 Inter-group comparison of BPA levels (mcg/dL) at T1.

Comparison of 30 minutes: Group1 Versus Group2				
T1 (30 min)	Sample	Mean	Standard Deviation	P-Value
Group1	20	0.02691213607	0.0009931571715063	0.0000<
Group2	20	0.02411914902	0.0006574946540779	0.05, S
There is a significant difference across the two groups				

By comparing the amount of BPA quantitatively among the samples collected at 1 week after curing and immersion in artificial saliva (T2), it was identified that Group 1 has comparatively more release and Group 2 has less release as shown in Table 11

The inter group comparison at T2 is listed below (Table 11)

Table 11 Inter-group comparison of BPA levels (mcg/dL) at T1.

Comparison of 1 week: Group1 Versus Group2				
T2 (1 week)	Sample	Mean	Standard Deviation	P-Value
Group1	20	0.07356302649	0.0009034870080501	0.0000<0
Group2	20	0.06725862487	0.0011546157778869	.05, S
There is a significant difference across the two groups				

By comparing the amount of BPA quantitatively among the samples collected at 1 month after curing and immersion in artificial saliva (T3), it was identified that Group 1 has comparatively more release and Group 2 has less release.

The inter group comparison at T3 is listed below (Table 12)

Table 12 Inter-group comparison of BPA levels (mcg/dL) at T1.

Comparison of 1 month: Group1 Versus Group2				
T3 (1 month)	Sample	Mean	Standard Deviation	P-Value
Group1	20	0.09551932813	0.0009091790270385	0.0000<
Group2	20	0.09134682738	0.0011930357769053	0.05, S
There is a significant difference across the two groups				

The overall comparisons of BPA levels, between the 2 groups have been tabulated statistically based on ANOVA and are shown in Table 13.

Table 13 Overall comparison of BPA levels (mcg/dL) at T1 &T2.

Duration	Group	Mean
30 minutes	Group1	0.027
30 minutes	Group2	0.024
1 week	Group1	0.074
1 week	Group2	0.067
1 month	Group1	0.096
1 month	Group2	0.091

DISCUSSION

The use of BPA in orthodontic treatments has drawn more caution from past few years due to its possible endocrine disrupting qualities. The effects of BPA exposure from orthodontic materials on patients have been studied, particularly in light of the extended periods of time patients spend in touch with these materials while receiving treatment.

In the field of dentistry, the two possibilities for BPA release from composites are during or shortly after resin installation, when incomplete monomer polymerization leads to the release, and later on, when material degradation takes place. Stefova M demonstrated that higher amounts of monomers were produced with less exposure to light, indicating that the 40-second exposure time recommendation should be followed.

Furthermore, reduced residual monomer levels were obtained when adhesives were used in conjunction with the recommended exposure period, highlighting the significance of adhesive use for full polymerization. (60) According to reports, BPA is completely eliminated after 42 hours, although there are still residues of the chemical present in the organs.

Also, there is a significant correlation between degree of cure (DC) and release of cytotoxic chemicals in Orthodontic adhesives. Insufficient curing leads to incomplete polymerization, leaving residual monomers and other harmful substances in the adhesive. Upon release into the oral cavity, these molecules can trigger adverse reactions, such as inflammation, allergies, and cell damage, especially in the delicate oral tissues. Thus, achieving an optimal degree of cure is vital to reducing the release of cytotoxic molecules from orthodontic adhesives, safeguarding the well-being of patients undergoing orthodontic treatment.

Various techniques, including Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), have been employed to detect hazardous compounds in orthodontic adhesives. HPLC, in particular, is valuable for separating and analyzing non-volatile and thermally unstable chemicals, which are common in adhesive formulations and challenging for gas phase chromatography. It allows for precise identification and quantification of cytotoxic compounds, confirming the safety and efficacy of orthodontic adhesives.

Furthermore, HPLC can analyze complex mixtures without the need for sample derivation, simplifying the analytical process and reducing the risk of sample deterioration.

This study utilized HPLC to investigate potential differences in the release kinetics of Bisphenol-A (BPA) from two composite adhesives when immersed in artificial saliva over varying durations.

Results showed a progressive increase in BPA release over time from both groups. In Group 1 (3M Concise Ortho), BPA release was 0.02691213607 mcg/dL at 30 minutes (T1), 0.07356302649 mcg/dL at 1 day (T2), and 0.09551932813 mcg/dL at 1 month (T3) post-curing and immersion in artificial saliva. Group 2 (Transbond XT) exhibited BPA releases of 0.02411914902 mcg/dL at T1, 0.06725862487 mcg/dL at T2, and 0.091334682738 mcg/dL at T3 after curing and immersion. These findings align with previous studies, highlighting the importance of understanding BPA release kinetics due to its potential health effects.

Intra-group analysis of Group 1 revealed significant temporal variations in BPA levels, emphasizing the need for monitoring over time. Differences between T1 and T3 were substantial, indicating a significant increase in BPA release over the study period. Conversely, differences between T2 and T3 were smaller, suggesting a more stable release rate later on. Understanding these variations is crucial for optimizing treatment protocols and reducing health risks associated with BPA exposure.

Further research is necessary to uncover the mechanisms behind these variations and develop strategies to mitigate BPA release from orthodontic adhesives. Group 2 also exhibited significant variability in BPA release over time, with the largest difference between T1 and T3, indicating a substantial increase over the study duration. However, the smallest difference between T2 and T3 suggested a more stable release rate later on.

These findings are consistent with several studies that have investigated the release of bisphenol A (BPA) from orthodontic

adhesives conducted by different authors, namely Eliades et al. (2011) Sunitha et al. Purushothaman (2015) Moreira (2017) & Hezenci.

The leaching of Bisphenol-A (BPA) from Transbond XT adhesive showed a progressive increase with longer immersion durations. These findings underscore the importance of understanding the release kinetics of BPA from orthodontic adhesives, as prolonged exposure to BPA has been associated with adverse health effects. However, the results of this study contradict those of an in vivo study by Kotyk et al. (2014), where BPA leaching was only observed at 3 days and 1 week, and not thereafter.

In the intra-group analysis of Group 2 using Transbond XT, the study revealed significant variability in BPA levels over time. The largest mean difference was found between T1 and T3, indicating a substantial increase in BPA release over the study period. Conversely, the smallest difference was observed between T2 and T3, suggesting a more stable BPA release rate in later stages. These differences were statistically significant, highlighting the temporal variability in BPA levels within the group.

The overall results of the study, after assessing BPA release at different time intervals post-curing and immersion in artificial saliva, showed that: At T1 (30 minutes post-curing), Group 1 (using 3M Concise Ortho.) exhibited the highest BPA release of 0.02691213607 mcg/dL.

At T2 (1 week post-curing), Group 1 continued to show the highest BPA release, with levels increasing to 0.07356302649 mcg/dL. Finally, at T3 (1 month post-curing), Group 1 maintained its lead in BPA release, reaching the highest level of 0.09551932813 mcg/dL.

Consistently, Group 1 (Concise Ortho), the chemical cure adhesive, released a higher amount of BPA than Group 2 (Transbond XT), the light cure adhesive, at all immersion times. Moreover, the maximum BPA release occurred at the 1-month mark for both groups, with Concise Ortho releasing more BPA than Transbond XT. This difference in BPA release could be attributed to several factors, including the higher levels of BPA typically found in chemical cure adhesives compared to light cure adhesives, as supported by previous studies.

The differences in manufacturing procedures and the degree of material degradation may be the cause of variations in BPA release at different time intervals among different orthodontic adhesives.

These variables may impact the composition and structure of the adhesive, influencing how BPA is incorporated into and released from the material. Understanding these variations is crucial for evaluating the potential health hazards associated with orthodontic interventions and underscores the importance of understanding the composition and properties of orthodontic adhesives and their potential health implications.

CONCLUSION:

The study provides valuable insights into the release kinetics of BPA from orthodontic adhesives, highlighting the importance of adhesive selection in orthodontic treatments. The findings suggest that chemical-cure adhesives may release higher levels of BPA compared to light-cure adhesives.

This information is crucial for clinicians and researchers in understanding the potential cytotoxic effects of orthodontic adhesives and for making informed decisions regarding adhesive selection in dental practice.

Further research is warranted to investigate BPA release in vivo and to understand its potential long- term effects on oral health. Additionally, larger sample sizes and longer follow-up periods are recommended to validate the findings of this study. Overall, this study contributes to the existing knowledge on the toxicity of resin-based materials and provides a foundation for future research in this field.