Increase of Fluoride Concentration on Mice Blood Plasma and Teeth after Consuming Bottled Water Fluoridation

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Abstract
Research aim to investigate the effect on the mice blood and teeth after consumption of fluoride bottled water.

Quasi-experimental research design with two groups of pure and adding fluoride of bottled water were made in the form of a sealed glass and stored at room temperature and exposed to sunlight. The in vivo test performed on two groups of Wistar mice to measure fluoride levels in blood plasma after consuming bottled water on days 1, 15 and 30, while the levels of fluoride on tooth measurements performed on day 30. ANOVA test was conducted to determine levels of fluoride in blood plasma.

There are differences in fluoride concentration in the blood of mice and mice teeth after consumption of bottled water and non-SnF. SnF bottled water consumption increases the concentration of fluoride in the blood and teeth.

Keywords: Fluoridated drinking water, fluoride concentration in blood plasma and teeth.

Introduction
Prevalence Caries tends to decrease in develop countries, meanwhile in developing countries increase.1 Roesdal found data 76,92% caries index: decay, missing and filling (DMF-T) on children aged 12 years old.2 Report from Indonesia Ministry of Health (Riskesdas 2008) stated 72,1% Indonesians has dental caries and 46.5% was active caries which has not been treated. Based on the data, caries prevention strategy is needed and the main priority is the effort to decrease caries prevalence.

Fluoride addition in the oral environment will increase remineralisation.3 Fluoridation can be applied topically and systemically. Fluoride is administered in low dose, regularly and through out life us the best method to increase tooth resistance towards caries attack.4 In this case, the use of fluoride toothpaste and fluoride in drinking water is the most appropriate method.

Water fluoridation effort in Semarang city, Central Java Province, Indonesia difficult to be implemented, as Local Government Water Supply (PDAM) which responsible for drinking supply in whole region is not willing to do with reason technical difficulties to provide fluoride and to maintain its stability and also economical problem related to high cost for consumer.5 An alternative of water fluoridation with low cost and technical is considered. Bottled water with fluoride added is one of the alternative.

Bottled drinking water is now become everyday need of the community as its practical and reasonably cost. A research about water fluoridation in bottled with concentration of SnF 0.7 ppm has been done with result that fluoride was stable after storage for 1, 4 and 8 weeks in glass with sealed and organoleptic test shown consumer acceptance on fluoride added in drinking water based on taste, smell and color.6

Fluoride will be absorbed into blood plasma and goes to teeth which a calcium rich tissue. Some of fluoride will be excreted via urine. To find affectivity of fluoride bottled drinking water after being consumed, an in vivo research need to be conducted on mice Wistar strain to determine fluoride concentration on
teeth and to find fluoride concentration on blood plasma.

**Materials and methods**

The research design was Quasy Experimental

Step one: prepared water glass fluoridation. Procedural of bottled drinking water manufacture are as follow: put raw water in the tank, added SnF until concentration reach 0.7 ppm, filtered it from the sand, actif carbón and micro filler, and final step was desinfectan and prepared the packaging.

Sample size for in vivo test was 1 ml of blood plasma of 5 Wistar strain mice after consuming fluoride drinking water for 30 days (the blood taken on day 1, 15 and 30) and for control group was 5 mice which drinking no fluoride water. Fluoride concentration on blood plasma on 1, 15 and 30 days after consuming bottled drinking water and fluoride concentration on teeth after 30 days consuming fluoride bottled drinking water. Control group was mice consume no fluoride bottled drinking water.

Procedure to determine fluoride concentration on blood plasma and teeth:

Prepared 5 mice weight ± 250 mg with diet ad libitum and drink with SnF drinking water 3 caps/day. Sample blood plasma 3 ml was taken at day 1, 15, 30 through vena orbitalis. Blood then stirred at Polietilen tube with natrium heparin and then centrifused with 1.000 times per minute for 15 minutes to separate the plasma. Sample was taken by dissolved 1 ml of plasma in 9 ml HClO₄ 0.5 M and 10 ml TISAB II. To determine fluoride concentration with Potensiometer Specific ion Fluoride.

Five mice with diet ad libitum and drink with fluoride drinking water for 30 days, the mice was euthanasia and tooth was extracted. Teeth are weighed, and then cut into small pieces, dissolved in 2.5 ml TISAB solution. Samples was 5 ml of teeth solution and concentration of fluoride was determined with Potentiometer Specific ion Fluoride.

To compare fluoride concentration on blood plasma after consume drinking water for 1, 15 and 30 days with Anova test. To find differences between treatment and control group on plasma blood and teeth, independent t test was used.

**Results**

After giving water contain fluoride for a month, a measurement of fluoride concentration on blood and teeth was done to find effect of drinking fluoride bottled water toward fluoride concentration on blood and teeth. As a control group was mice which drink water without fluoride. Below was picture of intervention group, blood collection from orbital vein and mice tooth extraction (Figure 1.).

![Blood collection from orbital vein and mice tooth extraction.](image)

**Figure 1.** Blood collection from orbital vein and mice tooth extraction.

<table>
<thead>
<tr>
<th>Drinking water</th>
<th>F (ppm) ± SD</th>
<th>Anova (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With F</td>
<td>0.3 ± 0.22</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>Without F</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Table 1.** Mean ± deviation standard of fluoride at mice blood after drinking water bottled fluoridation for 1, 15 and 30 days.

<table>
<thead>
<tr>
<th>Bottled drinking water</th>
<th>F concentration (ppm)</th>
<th>Independent t test (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF (Intervention)</td>
<td>2.24 ± 0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>No NaF (control)</td>
<td>1.8 ± 0.43</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Mean ± Standard Deviation (SD) of teeth fluoride concentration after drinking water fluoridation (intervention group) and control group for 30 days.

The table shown an increase of blood plasma fluoride concentration after drinking fluoride water for 1, 15 and 30 days, however fluoride enhancement was not significant difference (Anova p=0.15). Mice at control group which was given water without fluoride there was no fluoride at their blood plasma.
Discussion

There was a differences fluoride concentration on mice teeth which drink with fluoride bottled water and water without fluoride.

Test conducted on mice which drink bottled water fluoridation showed an increase in fluoride concentration in blood at measurement after days 1, 15 and 30 days and the increase was significant. The longer consumption water contain NaF, the concentration will increase.

Blood is medium for fluoride transportation before transfer to body tissue which need Calcium, namely bones and teeth. Calcified tissue at growth and development period will take fluoride in blood relatively greater than if the calcified tissue is mature enough in its growth.  

Range normal of fluoride level in blood plasma based on WHO (2002) is 0.4-2.4 μM (1 μM= 0.019 ppm). Fluoride blood level in this study was exceeded normal level of 0.8 ppm after consumption for 15 and 30 days. These results is different from study by 9 which found very low level of fluoride in the blood after put NaF patch for 1 week at mice skin.

The results showed an increase fluoride levels in blood after consumption of bottled fluoridation in weeks 1 and 4. The increase that occurred was not too high, proving the existence of a gradual increase in levels. These results are in accordance with a research which shows an increase in fluoride levels in the blood after the application of NaF patch although the increase is not high.  

Fluoride levels in teeth also showed very high results, even in the teeth of mice that consumed bottled water that did not contain NaF showed that fluoride levels were above 1 ppm. This is probably due to the relative age of the mice and has consumed foods containing fluoride, the second possibility is the instrument can not detect fluoride in low levels.

Based on the independent t test, there were differences in fluoride levels in teeth between those consuming NaF and non NaF bottled water. This shows the presence of fluoride in drinking water will increase fluoride levels in the teeth. There is no reference that states what is the right level of fluoride for teeth, because the level of fluoride in teeth will always change according to the intake. A former study found fluoride bottled water is appropriate to be produced by making proper packaging and storing.  

Fluoride levels in teeth are generally determined by several things: 1) age (the more a person ages, the fluoride levels in enamel increases; 2) fluoride intake during tooth growth, such as fluoride in drinking water or topical application of fluoride; 3) dynamic mechanism of fluoride absorption; 4) total substitution of hydroxyl groups from enamel with fluoride. The tooth acts as a fluoride reservoir, if it exceeds the required level, the tooth will not receive additional fluoride minerals. Fluoride needs of each element is different, if it is enough then the fluoride will be sent to other body parts that need or will be excreted.

Conclusions

Bottled water fluoridation is effective to increase fluoride concentration at blood and teeth.

Acknowledgements

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Declaration of Interest

The authors report no conflict of interest.

References