Role of acetyl salicylic acid to overcome toxicity of phenolic antioxidant (butylated hydroxy anisole) in albino rat.

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Abstract

Butylated hydroxy anisole (BHA) is a phenolic antioxidant. It is the most extensively used antioxidant in food industry as oil, margarine and butter. To determine the role of acetyl salicylic acid on reduction of BHA toxicity, thirty albino rats were divided into three groups. First group was fed on ration containing 1% BHA for two months. Second group was fed on ration containing 1%BHA and 0.2% acetyl salicylic acid in drinking water. Third group was kept as control. On comparison to the control group, BHA increased levels of AST, ALT, and deoxy ribonucleic acid and lowered levels of total protein, albumin and globulin. Macroscopic and microscopic lesions were severe in liver and stomach in first group. Such toxic effects were somewhat reduced but not prevented completely by addition of acetyl salicylic acid. These results suggest that acetyl salicylic acid reduces the conversion of tert butyle hydroquinone (TBHQ) to Tert Butyl Quine(TBQ) (metabolites of BHA). The latter is the more toxic but TBHQ also has some toxic effect.

Introductions

Butylated hydroxy anisole is a phenolic antioxidant used for many years as an additive to prevent oxidative spoilage of food and thereafter man is continuously exposed to them (WHO, 1987; Phillips, et al, 1989 and Whysner, 1993). However in addition to its action as an antioxidant, BHA exerts a wide variety of biological effects (Ito and Hirose, 1989). At low doses BHA inhibits carcinogen induced tumor development in rodents through induction of specific enzymes in the cytochrome P450 complex (Hocman, et al 1988 and Williams, 1986). At high doses BHA induced carcinomas in rat and hamster forestomach epithelium (Ito et al., 1983 a& b). Verhagen et al. (1989) mentioned that not only forestomach but also glandular stomach, intestine and esophageal tissues were susceptible to proliferative effect of BHA. BHA caused enlargement of liver (Altmann, et al, 1985) and proliferation of smooth endoplasmic reticulum and hepatocytic cytoplasmic myelinoid bodies (FAO/WHO, 1989). BHA capable of increasing 8-oxod G levels in liver cells (Schilderman, et al 1995).
In general the reactive forms of carcinogens are electrophilic species BHA by itself is not an electrophile (Schilderman et al. 1993a). Carcinogenic effect of BHA is due to the conversion of parent compound to more reactive metabolites. Butylated hydroxy anisole appeared to be carcinogenic to rat forestomach epithelium (Ito et al. 1983b). It caused squamous cell carcinomas in forestomach of rat and hamster (Altmann et al. 1985 and Hirsoe et al. 1986). BHA caused hyperplasia, papilloma and carcinoma (Nera et al. 1988 and Amo et al. 1990). These changes appeared to be dose and time dependent (Schilderman et al. 1992). Although the initial proliferative response as inflammation, hyperplasia and hyperkeratosis of the squamous epithelium is very rapid (Altmann et al. 1985), it takes a considerably longer period for carcinomas to develop (Masui et al. 1986). 1% tert-butyl hydroquinone in the diet of rats caused hyperplasia of the forestomach epithelium (Nera et al. 1984). 2%TBHQ caused brownish discolorations and mild hyperplasia of the forestomach mucosa with focally increased hyperplasia of basal cell (Altmann et al. 1985).

Although the toxic and carcinogenic effects of BHA are well described, little is known about the exact mechanism (Schilderman, 1993a). The irritating potential of BHA is not responsible for its carcinogenic action (Amo et al. 1990). The negative results in most tests for mutagenicity (Hoceman et al. 1988 and Rogers et al. 1985) suggest that BHA itself doesn’t react with DNA (Cummings et al. 1985). It is possible that the carcinogenic effects of BHA are due to the conversion of the parent compound to more reactive metabolites tert-butyl hydroquinone and tert-butyl quinone (Schilderman et al. 1993b). The main metabolic transformation is conjugation and 0-demethylation (Destaphney et al. 1986). The oxidative demethylation of Butylated hydroxy anisole occurred by cytochrome p450 and yeild tert-butyl hydroquinone (TBHQ) in vivo in dogs, rats and man (Astill et al. 1962 and Verhagen et al. 1989). TBHQ can be metabolized by peroxidases into tert-butyl paraquinone, which can generate active oxygen (Kahl et al. 1989 and Sakai et al. 1990). Therefor the known BHA metabolites (TBHQ & TBQ) are the active compounds which are capable of attacking macromolecules due to generation of active oxygen species (Destaphney et al. 1986).

DNA damage capacity of TBQ was much higher than that of TBHQ and BHA (Moromoto et al. 1991). However Schilderman et al. (1993b) mentioned that TBHQ but not BHA or TBQ appeared to be strong inducer of oxidative DNA damage owing to the generation of reactive oxygen species. Cells of the forestomach of rat taken BHA undergoing active DNA synthesis over 11 time higher than control animals (Newberne et al. 1986).

Administration of prostaglandin H1 synthase resulted in acceleration of tert – butyl hydroquinine (TBHQ) metabolism into tert – butyl quinine (TBQ) which was accompanied by hydroxyl radical formation. Such radical is suggested to be responsible for some toxic effect as damage to protein and DNA (Kappus, 1986). Administration of prostaglandin H synthase inhibition (acetyl salicylic acid & indomethacin) reduced the
proliferative effect of BHA (Schilderman, et al 1992). This work aims to evaluate the role of acetyl salicylic acid in prevention of BHA toxicity.

**Material And Methods**

A) Butylated hydroxy anisol (BHA) was obtained from Kamena products corporation Cairo, Egypt.

B) Acetyl salicylic acid was obtained from ARAB Drug CO. Cairo.A.R.E.

**Animals:** Thirty male Albino rats weighting from 100-120 g obtained from laboratory animal house Faculty of Veterinary Medicine Moshtohor. Rats were kept under hygienic conditions and fed on balanced rations and water ad-libitum. Rats were divided into three groups each of ten rats.

**Experimental design:** First group: Rats in this group were fed on ration contained 1% BHA according to (Ito et al, 1983b) daily for two months.

Second group: Rats in this group fed on ration containing 1% BHA and drinking water containing 0.2% acetyl salicylic acid according to (schilderman et al, 1993b) daily for two months.

Third group: Rats in this group were kept as a control. Rats from all groups were slaughtered after 2 months (end of the experiment).

**Samples:** Blood was obtained from slaughtered rats and allowed to clot, then serum was derived. Serum values of aspartate amino transferase and alanine amino transferase were determined using kits according to Reitman and frankel (1957). Serum total protein, albumin and globulin were determined according to Doumas et al (1981), Pinell and Northam (1978) and Coles, (1974) respectively. Total deoxy ribonucleic acid in liver and stomach tissues was determined according to Melmed et al (1975) (and Abdel Salam (1983). Serum level of butylated hydroxy anisol and its metabolite tert butyl hydroquinone were determined using HPLC according to Schilderman, et al, (1993a). Specimens from liver and stomach were collected and fixed in 10% formaline solution for histopathological examination according to Dury & Wallington, (1980).

Statistical analysis: The data obtained were collected as means ± standard error and statistically analyzed by student’s (t) test according to Johnston, (1972).

**Results**

During the experimental period no death were recorded among rat in all groups. The effect of butylated hydroxy anisol only or in combination with acetyl salicylic acid on alanine amino transferase (ALT) or aspartate amino transferase (AST) was recorded in Table (1). and Fig.(1) significant increase in the levels of ALT were recorded in first and second group in comparison to the control one. Values of serum ALT were 64.35±3.75, 58.9±2.3 u/ml for first and second group in comparison to 51.4±1.36 u/ml of control group. Serum values of aspartate amino transferase were increased in first group in comparison to the control one. Group fed on ration containing 1% BHA and 0.2% acetyl salicylic acid in drinking water showed no significant variation in values of serum AST (U/ml) in comparison to the control one.
Table (1): Effect of 1% butylated hydroxy anisol in ration and 1% butylated hydroxy anisol in ration with 0.2% acetyl salicylic acid in drinking water daily to albino rats for 2 months on serum ALT and AST (U/ml) compared to control group (Mean ± S.E).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>ALT(U/ml)</th>
<th>AST(u/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1% BHA in the ration</td>
<td>± 64.35**</td>
<td>± 64.74*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 3.75</td>
<td>± 7.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>1% BHA in ration and ASA in drinking water</td>
<td>± 58.9*</td>
<td>± 51.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 2.3</td>
<td>± 2.7</td>
</tr>
<tr>
<td>Group 3</td>
<td>Control group</td>
<td>± 51.4</td>
<td>± 43.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.36</td>
<td>± 2.4</td>
</tr>
</tbody>
</table>

* Significant at p< 0.05
** High significant at P<0.01

Fig. (1): Effect of 1% BHA and 1% BHA with 0.2% acetyl salicylic acid in drinking water for 2 months on serum ALT & AST (u/ml) compared to control group.

Table (2) illustrates significantly decreased levels in total protein of the first and second groups in comparison to the control one. Decrease in albumin level of the first group (fed on ration containing 1% BHA) was significantly in comparison to the control one. Albumin level in the first group was 2.94± 0.14 g/dl compared to 3.9± 0.52 g/dl of the control one. Serum globulin levels were significantly decreased in both first and second groups in comparison to the control one. Globulin levels were 1.86± 0.32 and 1.78±0.29 g/dl in the first and second groups respectively in comparison to 3.6±0.2 g/dl in the control one.
Table(2): Effect of 1% butylated hydroxy anisol in ration and 1% butylated hydroxy anisol in ration with 0.2% acetyl salicylic acid in drinking water daily to albino rats for 2 months on serum total protein, albumin and globulin (g/dl) compared to the control group (Mean + S.E).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Total protein (gm/dl)</th>
<th>Albumin (gm/dl)</th>
<th>Globulin (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>1% BHA in the ration</td>
<td>4.8**</td>
<td>2.94**</td>
<td>1.86**</td>
</tr>
<tr>
<td></td>
<td>± 0.27</td>
<td>± 0.14</td>
<td>± 0.322</td>
<td></td>
</tr>
<tr>
<td>Group2</td>
<td>1% BHA in ration and ASA in drinking water</td>
<td>5.3**</td>
<td>3.52</td>
<td>1.78**</td>
</tr>
<tr>
<td></td>
<td>± 0.23</td>
<td>± 0.35</td>
<td>± 0.29</td>
<td></td>
</tr>
<tr>
<td>Group3</td>
<td>Control group</td>
<td>7.54</td>
<td>3.9</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>± .38</td>
<td>± 0.52</td>
<td>± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p< 0.05  
**High significant at p<0.01

Deoxy ribonucleic acid content in liver and stomach tissues were recorded in Table (3) and Fig.(2). Total (DNA) content in liver of the first group fed on ration containing 1%BHA was significantly higher than that of the control one. DNA levels were 2.1± 0.18 and 1.36 ± 0.073mg/ml in liver of the first and control groups respectively. Total DNA content in stomach of the first group was significantly higher than that of the control one. Total DNA contents were 2.36± 0.16 mg/g for the first group on comparison to 1.29± 0.08 mg/g of the control group. DNA content in liver of the second group showed non significant variation in comparison to the control one. A significant increase in total DNA content of stomach of the second group was recorded in comparison to that of the control one.
Table (3) Effect of 1% butylated hydroxy anisol in ration and 1% butylated hydroxy anisol in ration with 0.2% acetyl salicylic acid in drinking water daily to albino rats for 2 months on total deoxyribonucleic acid(mg/g) content compared to the control group(Mean ± S.E).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>DNA(mg/g) in liver wet tissue</th>
<th>DNA(mg/g) in stomach wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>1% BHA in the ration</td>
<td>2.1** ± 0.18</td>
<td>2.36** ± 0.16</td>
</tr>
<tr>
<td>Group2</td>
<td>1% BHA in ration and ASA in drinking water</td>
<td>1.32 ± 0.025</td>
<td>1.9* ± 0.08</td>
</tr>
<tr>
<td>Group3</td>
<td>Control group</td>
<td>1.36 ± 0.073</td>
<td>1.29 ± 0.08</td>
</tr>
</tbody>
</table>

*Significant at p< 0.05  
**High significant at P<0.01

Fig.(2): Effect of 1%BHA in ration and 1% BHA in ration and 0.2% acetyl salicylic acid in water on DNA content of liver and stomach wet tissues.

Table(4) and Fig.(4&5) showed serum level of butylated hydroxy anisole showed non significant variation between first and second group .Serum tert butyl hydroquinone showed a significant increase in the second group (fed on ration containing 1%BHA and 0.2% acetyl salicylic acid in drinking water )in comparison to the first group fed on ration containing 1%BHA only. Values of serum tert butyl hydroquinone were 20.03± 1.44ug/l and 30.19 ± 2.47 ug/l for the first and second groups respectively.
Table(4): Effect of 1% butylated hydroxy anisol in ration and 1% butylated hydroxy anisol in ration with 0.2% acetyl salicylic acid in drinking water daily to albino rats for 2 months on serum butylated hydroxy anisol (BHA) and tert butyl hydro quinon (TBHQ) (Mean ± S.E).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>BHA (µg/l) in serum</th>
<th>TBHQ (µg/l) in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1% BHA in the ration</td>
<td>556.62</td>
<td>20.025 ± 1.44</td>
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<td></td>
<td></td>
<td>± 102.6</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>1% BHA in ration and ASA in drinking water</td>
<td>883.04</td>
<td>30.19** ± 2.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 144.7</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p< 0.05
**High significant at P<0.01

Fig.(3): Butylated hydroxy anisol (BHA) and tert butyl hydroquinon (TBHQ) standered
Macroscopic examination of the liver from rats fed on ration containing BHA showed different congested areas as clear in Fig (6) in comparison to less affected liver in rats fed on BHA and acetyl salicylic acid in drinking water or that of the control group as clear in Fig. (7).

Histological examination of the liver from rats of the first group showed large cell with hyperchromatic nuclei (Fig,8) and congested blood vessels with cellular infiltration as clear in (Fig,9).Liver from second group showed congested blood vessels and cellular infiltration as clear in Fig.(10&11) in comparison to the control group as clear in Fig.(12).Stomach from rat fed on ration containing 1% BHA showed epithelial cell proliferation as clear in Fig.(13) and cell proliferatin as a finger like as clear in Fig.(14&15) while the group fed on ration containing 1%BHA and 0.2% acetyl salicylic acid in drinking water showed epithelial cell proliferation with congested blood vessel as clear in Fig.(16)in comparison to the control group as clear in Fig.(17).
Fig. (6): Different congested areas of liver of rats fed on ration containing 1% BHA.

Fig. (7): Less affected liver of rats fed on ration containing 1% BHA and 0.2% acetyl salicylic acid in drinking water and that of the control group.

Fig. (8): Large cell with hyperchromatic nuclei in liver of rats fed on ration containing 1% BHA. Stain (H&E) X 200

Fig. (9): Congestion of blood vessel and cellular infiltration in liver of rats fed on ration containing 1% BHA. Stain (H&E) X 100
Fig. (10): Congested blood vessel and cellular infiltration in liver of rats fed ration contain 1% BHA & 0.2% ASA in drinking water. Stain (H&E) X 100

Fig. (11): Congested blood vessel in liver of rats fed ration contain 1% BHA & 0.2% ASA in drinking water. Stain (H&E) X 100

Fig. (12): Liver of control group. Stain H&E X 100

Fig. (13): Epithelial cell proliferation in stomach of rats fed on ration containing 1% BHA. Stain H&E X 100
Fig.(14&15): Epithelial cell proliferation finger like in stomach of rats fed on ration contain 1%BHA . Stain H&E X 100

Fig.(16): Epithelial proliferation and congested blood vessels stomach of rats fed on ration containing 1%BHA & 0.2% ASA in drinking water. Stain H&E X 100

Fig.(17): Stomach of control group. Stain H&E X 100
Discussion

Synthetic antioxidant is a phenolic substances widely used as additive in foods especially oils and fats in order to delay or prevent oxidative deterioration (Andrikopoulos et al., 1991). In various countries restriction exist concerning the use of antioxidants (Page, 1983). In this study rats fed on ration containing 1% BHA showed increased serum levels of ALT & AST. These results may be attributed to interaction of BHA metabolites (tert butyle hydroquinone & tert butyle quinone metabolites) with NADPH cytochrome C P450 reductase that caused disturbance in hepatic functions which severely increased the activities of transaminases (Ziegler-Skilakakis and Andro, 1987). TBHQ and TBQ are the active compounds, which are capable of attacking macromolecules and generation of active oxygen radicals (Destaphney, et al. 1986). At normal condition, the deleterious effects of oxygen radicals are balanced in tissues. However the production of these radicals increased or the host oxidant defenses are impaired excessive tissue injury results and physiological function of cells and organs are altered (Fantone and Ward, 1985). Such alteration in physiological function of the cells explains the increased serum level of ALT and AST. These results agree with Gaunt et al (1985); Benson, et al (1987) and Cha and Heine, (1982) who reported that BHA alters the activity of a range of hepatic enzymes. Also pathological lesion of liver as hyperchromatic nuclei, congested blood vessels and cellular infiltration may be augment these results. Such enzymatic variation and pathological lesion of the liver may be due to the reactive oxygen which initiates and potentates inflammatory responses through direct toxic effect on cells and modification of serum protein and structure component of tissue. On the other hand group of rats fed on ration containing 1% BHA and 0.2% acetyl salicylic acid in drinking water, showed increased level of serum ALT in comparison to the control one. Acetyl salicylic acid one the prostaglandin H synthase inhibitor that used to decrease the conversion of tert butyl hydroquinone to tert butyl quinone (Schilderman, et al 1993b). The latter (tert butyl quinone ) has toxic effect more than BHA and TBHQ (Moromoto, et al, 1991). However an increase in serum ALT was recorded as a result of tert butyl hydroquinone toxicity as mentioned by Nera, et al,(1988). This result agrees with Schilderman et al,(1993b) who found that TBHQ, but not BHA or TBHQ appeared to be strong inducer of oxidative DNA damage in vitro, owing to the generation of reactive oxygen species . It is possible that oxygen radical directly react with intracellular macromolecules such as thiol groups of enzymes. These results are augmented by
pathological lesion of liver in the form of congestion of blood vessel and cellular infiltration as clear in Fig.(10&11).

Serum total protein, albumin and globulin were decreased in the group fed on ration containing 1% BHA. These results may be explained by BHA metabolites(TBHQ & TBQ) react with thiols and NADPH in the protein of the tissues (Phillips, et al1989). Also reactive high level of covalent binding of BHA to protein was detected (Hirose, et al., 1987). Decreases in serum total protein and globulin were detected in group receiving 1%BHA and acetyl salicylic acid. These results may be attributed to toxicity of tert butyl hydroquinone, which increased in blood as a result of administration of prostaglandin H synthase inhibitor (acetyl salicylic acid) as clear in Table (4). TBHQ is an active compound which is capable of attacking macromolecule due to generation of active oxygen (Destaphney, et al 1986). Pathological lesion of liver in the form of congestion and cellular infiltration augmented these results.

The increased levels of total DNA content in liver and stomach content were detected in the group fed on ration containing 1%BHA. This result agrees with Newberne, et al, (1986) who mentioned that cells of forestomach undergoing active DNA synthesis over 11 times higher than in control animals. In the second group, total DNA level increased only in stomach tissue. This result may be due to TBHQ toxicity. The increased total DNA level in the first and second groups might be related to proliferation of tritiated thymidine (specific DNA precursor) as reported by Nera, et al (1984) and stimulation of DNA synthesis resulting in an increase in mitotic index (Walker and Quattrucci,1988).This effect is mediated by the parent (BHA) or metabolite compounds (TBHQ&TBQ)(Walker and Quattrucci, 1988 and Thompson, et al ,1989).These results are augmented by serum level of TBHQ and pathological lesion especially that of stomach.

Non significant variations in serum BHA levels were detected between the first and second groups. tert butyl hydroquinone in the second group was increased in comparison to the first one. This results may be attributed to inhibition of prostaglandin H synthase that has a role in the conversion of TBHQ to the metabolite tert butyl quinone by acetyl salicylic acid (Schilderman , et al 1993b). Although decrease in the conversion of TBHQ to TBQ and so release of oxygen radical was decreased, toxicity of BHA not prevented completely where formation of TBQ was not prevented completely and TBHQ still caused adverse effect as reported by Nera, et al (1984),Altmann, et al, (1985) and Moromoto, et al (1991).This explanation was augmented by pathological lesion of the liver and stomach as in Fig. (10,11&16) which is less severe than that of the first group.
Post mortem examination of liver showed different areas of congestion which are more severe in the first group as clear in Fig. (6). This results may be attributed to the toxic effect of BHA metabolites as TBHQ & TBQ. These results were augmented by lesion of the liver in this group. Lesion of the liver in the second group is less severe. These results may be due to inhibition of prostaglandin H synthase mediated metabolism of TBHQ into TBQ and so release of reactive oxygen (Schilderman et al.1993b). Stomach from rats fed on ration containing 1% BHA showed epithelial cell proliferation more severe than that of the second group. This result agrees with Clayson et al. (1986), Hirose, et al (1987) and could be attributed to the irritant effect of BHA on the stomach epithelial tissue as mentioned by Schilderman at al. (1995) in addition to the toxic effect of TBHQ and TBQ (metabolites of BHA).

References


Role of acetyl salicylic acid


FAO/WHO (1989):”Toxicological evaluation of certain food additives and contaminant” prepared by the 33rd Meeting of the joint FAO/WHO Expert Committee on food additives. Geneva.21-30 P.14


covalent binding to tissue macromolecules.
Toxicology.45: 13-24


دور خفض تفاعلات الغلي介绍 ساليسلية في خفض سمية مضادات الأكسدة
(البيوتينيل هيدروكسي أنيسول) في الفئران البيضاء

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نظراً للاستخدام الواسع لمضادات الأكسدة أصبح من الضروري محاولة السيطرة على آثارها السامة. لذلك هدف الدراسة هو تقديم دور حمض الأمينوتيل ساليسلية (والمرحوم بدوره في خفض تكوين البروتستاجلاندين والذي له دور في تحويل مادة البيوتينيل هيدروكسي أنيسول إلى مشتقات عالية السمية وخروج الأسنان الحرة والتي تحدث تأثيرات سامة خاصة على الكبد والجهاز الهضمي وعلى الأخضر المدة (لذلك أجريت الدراسة على عدد ثلاثون فارين قسمت إلى ثلاث مجموعات تغذت المجموعة الأولى على علبة تحتوي 1% بيوتينيل هيدروكسي أنيسول. أما المجموعة الثانية فنفّذت على نفس الطريقة بالإضافة إلى 2،0% من حمض الأمينوتيل ساليسلية في مياه الشرب يوميا لمدة شهرين متتاليين وترك المجموعة الثالثة كمجموعة ضابطة. ذكفت الفئران في ناحية السمنة وفرص المصل. تم دراسة مدى تأثر الكبد واللثة عند طريقة قبض مستوي أنزيم إنزيمات الأحماض النووية والبروتين الدهني والبروتين الكلي والألبومين وكذلك الجلوبيولين كما تم قياس كمية الحمض النووي الميزوكيزي في نسيج الكبد والمعدة. تم قبض مستوي طريقة تبرت أحيائي هيدروكسيون أحد مشتقات مادة بيوتينيل هيدروكسي أنيسول. كما قمت الدراسة التغيرات البيولوجية في الكبد والمعدة أوضحت النتائج أن حمض الأمينوتيل ساليسلية قد أحدث ازداد في سمية بيوتينيل هيدروكسي أنيسول على طريقة تراكم مادة تبرت أحيائي هيدروكسيون وعند التحلالات تبرت أحيائي كيبي. وقد ظهر ذلك في بعض النشاطات الكلية وكذلك الألياف وانخفاض كمية الحمض النووي الميزوكيزي في نسيج الكبد وكما ظهر ذلك التغيرات البيولوجية في الكبد والمعدة والتي كانت أكثر وضوحًا في المجموعة الأولى ولكنها لم تتفق تمامًا في المجموعة الثانية. وقد يرجع ذلك إلى أن حمض الأمينوتيل ساليسلية لم يمنع تأثير تبرت أحيائي هيدروكسيون على سمية استفادة