

Study Of Some Enzymatic And Histopathological Variants Of The Effect Of Sodium Benzoate On Rabbits

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Abstract: *This study was conducted in 2019-2020 for the purpose of determining the histopathological effects of sodium benzoate on the liver and kidneys and the effects on the level of enzymes for ALT and AST, where twelve rabbit eggs were used. Where the animals were divided randomly into three groups, namely: the control group: - which includes four rabbits and the first treatment group N1 (oral dose with sodium benzoate at a concentration of 100 mg / kg of body weight) and the second treatment group N2 (oral dose of sodium benzoate at a concentration of 200 mg / (Kg), where the totals were dosed orally daily for a period of 30 days.*

The animals were killed after the end of the experiment and then the liver and kidneys were removed for the purpose of conducting a study of the changes in them. These changes appeared in the liver tissue represented by the presence of hemorrhage and lymphocyte infiltration with the rupture of the cells lining the central vein, and there were also a few cases of karyolysis with the presence of some nuclei that Small size and condensation suffered by Pyknotic Nuclei.

In the kidney tissues, the histological lesions were represented by a general necrosis of the tissue, Necrosis and Vacuolated Necrosis, with the presence of degeneration within a section of the glomeruli within the cells that made it, with the observation of dense and small nuclei, which is on the way to death.

As for the enzymatic effects, the results of the statistical analysis indicated the presence of significant differences ($P < 0.05$), the level of ALT and AST enzymes between the two concentrations of sodium benzoate when compared, while the level of enzymes increased significantly ($P < 0.05$) with increasing concentration.

INTRODUCTION

Where benzoic acid, which is an organic acid, as well as its salt, sodium benzoate, are commonly used as preservatives for many products consumed by humans (FDA.2011). The maximum permissible limits for benzoate in food vary, reaching 0.1% in the United States of America, while in other countries of the world it ranges between 5-0.015.0% (European Commission 1995). Several studies were conducted aimed at investigating the short and long term effects of consuming products preserved with benzoate, and most of these studies dealt with organ organization and clinical criteria for both humans and experimental animals. Some of these studies indicated the presence of adverse effects due to both chronic and sub-chronic consumption of sodium benzoate, including Changes in serum parameters, relative

increase in kidney and liver weights, histopathological changes in the liver, and disorders related to the central nervous system (Fujitani., 1993) (Vogt, et al., 1999).

Food Additives

Chemical preservatives are used to prevent chemical and biological spoilage of food. Chemical spoilage includes oxidation and browning of food. Bio-spoilage involves the decomposition of food by the action of microorganisms. Among the chemical preservatives are anti-microbial preservatives that inhibit the growth of bacteria and fungi, which can produce undesirable effects on both the appearance and taste of food as well as its nutritional value, and it can also produce toxins that pose a great danger to human health, and examples of these preservatives are sodium benzoate (Nishnaet al., 2012)

Sodium benzoate is produced from the neutralization of benzoic acid with sodium hydroxide, which in turn produces benzoic acid if it is dissolved in water, since although the benzoic acid is dissolved in water, it is more effective for preservation purposes, but it is preferable to use sodium salt for benzoic acid (sodium benzoate) to preserve food for its dissolution in Water is about 200 times more than benzoic acid. Sodium benzoate has anti-microbial efficacy such as fungi and some types of bacteria, and its use as a food preservative is ideal in products that are acidic in nature, especially food and beverages with a pH > 4.5, (Stanojevic, et al 2009)

The chemical composition of the compound and some of its properties:

Sodium benzoate:

A chemical compound with the molecular formula C_6H_5COONa , which is the sodium salt of benzoic acid. It is used in food additives and has the number (E211). Srour, 1998.((

Properties

The solubility of sodium benzoate is good in water, about 660 g / l.

The compound is odorless and dissociates under heating. (Heydaryinia, et al ., 2011).

General physiological effects of benzoates

In a study conducted on rats, it was found that benzoic acid and sodium benzoate were high (800 mg / kg for benzoic acid and 1000 mg / kg for sodium benzoate), which leads to an increase in mortality and a decrease in weight, resulting in systemic toxicity and damage to the liver and kidneys.

In a scientific study conducted on mice, it confirmed that high doses affect body weight and cause lesion erosion in the brain, thymus gland, skeletal muscles and kidneys.

In rabbits, it was found that exposure to undiluted benzoic acid causes irritation to rabbits' eyes, and exposure to sodium benzoate causes only slight irritating slightly.

Some studies indicated that after short-term treatment with sodium benzoate and benzoic acid, pathological changes were observed in the brain in rats in addition to disturbances in the central nervous system.(Sinha and, souza (2006) stated that sodium benzoate if given to mice for 30 periods of time. , 60, 120, 180 days, at a concentration of 155 mg / kg, led to an increase in the weight of the kidneys and liver, decrease in their body weight, length of the spleen, and a significant increase in the level of protein, globulin and albumin.

While another study showed that oral administration of sodium benzoate at a concentration of 280,560 mg / kg to white female mice for a period of 60 days led to a significant decrease in the level of progesterone in the blood plasma, in addition to a significant decrease in the levels of the Luteinizing hormone LH and FSH in the group that was administered. Given a concentration of (560) mg / kg, it was found that benzoate, at a concentration of 200 mg / kg, can reduce weight in mice and increase creatine, urea and uric acid in serum isolated from mice. It was observed that sodium benzoate had an effect on increasing blood pressure in rats and causing rupture in Vascular cells: During the study of lymph node cells isolated from mice and treated with different concentrations of benzoate, and compared with control cells, it was found that benzoates can alter lymphocyte structures and cause damage to the cell membrane with high concentrations and the time of exposure to this substance increases the negative effects. Studies have shown that benzoates have an effect on Reducing white blood cell counts and hemoglobin in rats when treated with benzoates at a concentration of 60,120 mg / kg compared with a control group (Sohrabi, et al ., 2008) .

THE METHOD OF WORK

Experimental distal design experiment

Sodium benzoate was prepared according to the method (Sabr and Ibrahim, 2015)

After obtaining 12 of the laboratory rabbits, it was divided into four groups, where each group consists of four rabbits, and these groups are:

The first group: This group included 4 animals that were dosed at a dose of 100 mg / kg of body weight for 30 days, and after the end of the dosing period, the animals were sacrificed and the liver and kidneys were removed for the purpose of histological study.

The second group: This group included 4 animals that were dosed at 200 mg / kg of body weight for 30 days, and after the end of the dosing period, the animals were sacrificed and the liver and kidneys were removed for the purpose of histopathology.

The third group: This group included 4 animals dosed with a physiological saline solution at a concentration of 0.9% for 30 days. After the end of the dosing period, the animals were sacrificed and their internal organs removed, which included the liver, heart and bone marrow, for the purpose of histological study.

These rabbits were dosed in the previous groups orally with sodium benzoate solution prepared in the laboratory for a period of thirty days at a rate of (1 cc) per 24 hours.



RESULTS AND DISCUSSION OF THE STUDY

The metabolism of sodium benzoate

The process of metabolism of this compound is carried out by living organisms and it can ultimately make an active compound that interacts with DNA and changes the genotype and has negative effects on cell division. Metabolism is carried out in the mitochondria of liver cells by attaching benzoates to the amino acid glycine and expelling it outside the body in the form of hippuric acid. From diuresis, the natural secretion of hippuric acid in the urine daily, and when consuming foods containing benzoate daily, this amount increases.

Those procedure for discharge from claiming glycine from the form demonstrates impedance done liver capacity in the metabolic forms within which glycine is essential. Previously, addition, low levels about glycine in the constitution might diminish levels of creatine, glutamine, urea and uric corrosive in the pee What's more increment the levels about these substances in the blood, hydrogen bonds. Hydro phobic ought further bolstering make successful done tying benzoate with glycine aminic acid, and the amount about sodium benzoate if a chance to be addition.

With this connection, Previously, An investigation led Previously, 2012 around 3083 belgian consumers, it might have been closed that these individuals utilization 1. 25 mg / kg. From claiming sodium benzoate for every day and this measure will be 0. 25% of the adequate Every day limit, which may be addition should tie benzoates for 50 mg about glycine Furthermore is excreted in the type for hippuric corrosive. It need a paramount part in the assimilation from claiming nourishment results and The point when interfaced should benzoates, this prompts the restructuring about trypsin. (Oyewole et al. 2012).

Effect on enzymes

Table (1) The effect of different concentrations of sodium benzoate on the concentration of ALT (L / U) and AST (U / L) in adult rabbits.

| ALT | AST | enzymes | Groups |
|------------------|-------------------|---------|--------|
| A 0.545±23.86 | A 0.583± 34.22 | | C |
| B 0.735±34.20 | B 0.613± 39.26 | | N1 |
| C 0.548±38.04 | B 0.795± 41.92 | | N2 |

The numbers indicate the rate = standard error.

The different capital letters indicate that there are significant differences (P <0.05) between the concentrations. C represents the control group.

N1 was the first group dosed with sodium benzoate at a concentration of 100 mg / kg

N2 represents the second group that was dosed with sodium benzoate at a concentration of 200 mg / kg

1- The effect on the level of the enzyme ALT

The results shown in Table (1) indicated that the treatment of adult rabbits in the first and second groups with sodium benzoate at concentrations of 100 and 200 mg / kg of body weight respectively for 30 days resulted in a significant increase ($P < 0.05$) in the level of the ALT enzyme in the blood serum. For the above totals compared to control totals.

The results of the statistical analysis shown in Table (1) indicated that there were significant differences ($P < 0.05$), the level of ALT enzyme between the groups treated with the three concentrations of sodium benzoate when compared, while the level of the ALT enzyme increased significantly ($P < 0.05$) with the increase in concentration. (Table 1)

2- The effect on the level of AST enzyme

The results showed a significant increase ($P < 0.05$) in the level of AST enzyme in the blood serum of adult rabbits in groups treated with sodium benzoate at concentrations of 100 and 200 mg / kg of body weight respectively for 30 days compared to the control group. The results also indicated that the N1 and N2 group showed a significant decrease ($P < 0.05$) in the level of AST enzyme compared to the control group. (Table 1)

The results of the statistical analysis also indicated that there were significant differences ($P < 0.05$) in the level of AST enzyme between the groups treated with concentrations of sodium benzoate if the level of AST increased significantly ($P < 0.05$) depending on the increase in the concentration of sodium benzoate.

3- The effect on the level of the enzymes ALT and AST

Liver enzymes are clinically important as their rate of effectiveness is dependent on cellular damage that leads to their release into peripheral fluids and then into the blood, including ALT and AST enzymes. The ALT enzyme is produced mainly in the cytoplasm of hepatocytes, so it is the most specialized in detecting Liver diseases are also found in other tissues, but in trace amounts, such as skeletal muscles, heart, kidneys, pancreas, spleen, lung and blood serum.

As for AST, it is considered a mitochondrial enzyme mainly due to its presence in it, in addition to its presence in other organs such as the heart, skeletal muscles, kidneys, brain and red blood cells. Accordingly, their concentration in the blood gives a picture of the extent of their activity in those organs, especially the liver (Dufour et al., 2000.)

The results of the current study showed that treatment of adult rabbits with increased concentrations of sodium benzoate resulted in a significant increase in the levels of these enzymes in the blood serum compared to the control group. The results of the current study can be attributed to the fact that these enzymes were leaked in large quantities from liver tissue into body fluids, especially serum, and that this high leakage reflects the extent of damage to body tissues, especially the liver. These results reinforce previous studies that indicated the damage caused by sodium benzoate to liver tissue, which is the direct toxic effect on hepatocytes and their degeneration (Sinha et al., 2010) (Khidr. Et al 2012)

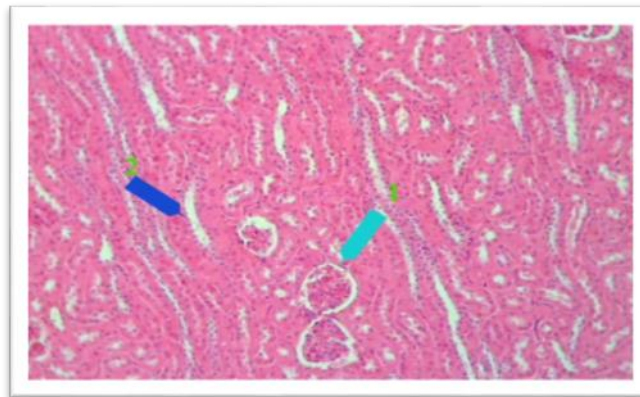
Which leads to the leakage of its contents, including these enzymes, into the circulation, as the liver is one of the largest organs specialized in performing a variety of functions in the

body, including removing toxins, which makes it vulnerable to damage due to these substances and what is produced from their metabolism in it (Guyton et al 2011). The activity of amine transporting enzymes is used as a sensitive measure of the extent of pathological and physiological changes that require additional activity in the metabolic processes by the liver (Ozer J et al 2008).

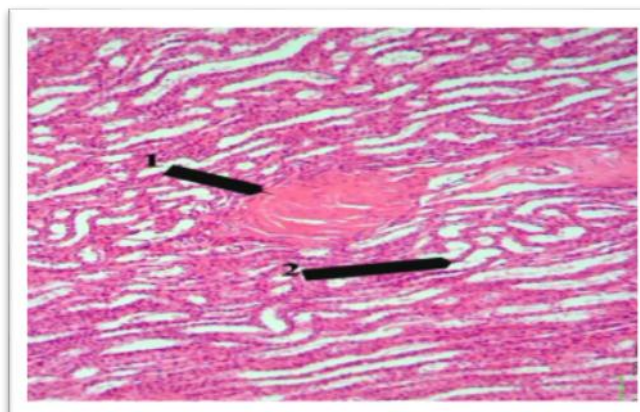
The rise in ALT and AST levels can also be attributed to the formation of free radicals that attack the plasma membranes of liver cells, which leads to the leakage of these enzymes as the state of oxidative stress resulting from an increase in active oxygen levels is a cause of the breakdown of DNA, proteins and fats in hepatocytes, which leads to This leads to the degeneration and destruction of these cells and then exudation of their contents into the bloodstream, including the enzymes ALT and AST. (Lu. Et al 2010).

With regard to the effect of the duration of dosing, the results showed that the greater the duration of the dosing with sodium benzoate, which led to an increase in the levels of these enzymes. This may be attributed to the fact that benzoate is one of the substances whose effects are cumulatively evident in the body and its effect increases with the increase in the given concentration and the duration of the dose.

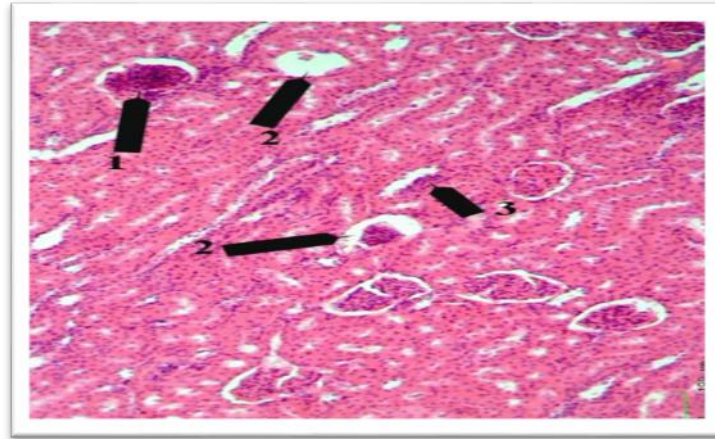
4- Histopathological changes



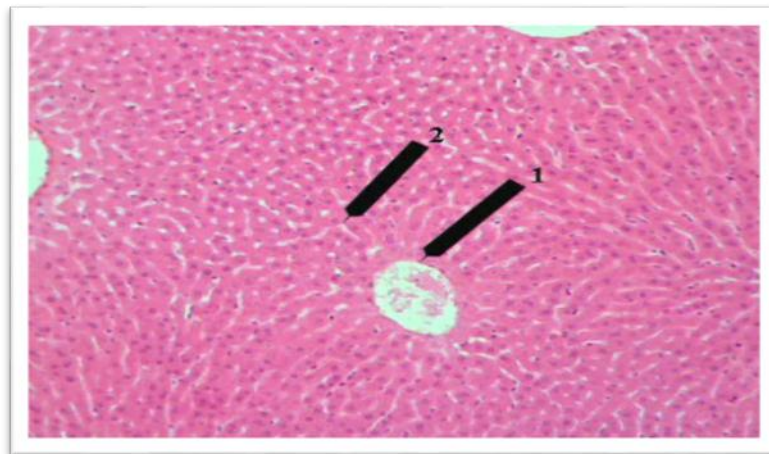
Picture (1) rabbit kidney control group showing (1) renal glomerulus 2) renal tubules H&E , 400 X



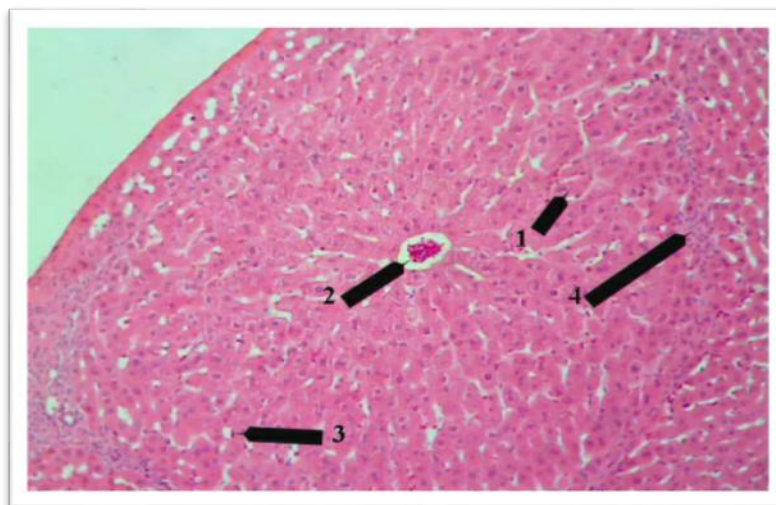
Picture (2) Rabbit kidney with a dose of N1 sodium benzoate (1) Congestion Con. 2) Rupture of the renal tubules H&E, 400X.



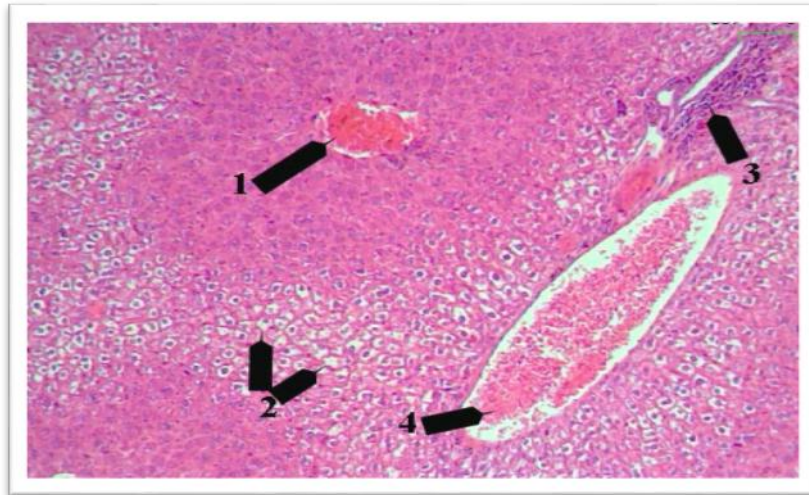
Picture (3) Rabbit kidney with a dose of N2 sodium benzoate (1) congestion in the glomerulus. 2) necrosis in the renal glomerulus. 3) infiltration of inflammatory cells (IF) H&E, 400X



Picture (4) Rabbit liver at a dose of N1 sodium benzoate 1) Central vein congestion, 2) Sinisoid . H&E, 400 X



Picture (5) rabbit liver with a dose of N1 sodium benzoate 1) cirrhosis of hepatocyte F, (2) congestion in central vein Con, 3) Kupffer cells 4) infiltration of inflammatory cells H&E, 400X



Picture (6) rabbit liver with a dose of N1 sodium benzoate 1) central vein hemorrhage, He, 2) cytoplasmic necrosis, 3) infiltration of inflammatory cells, 4) rupture of H&E central vein wall, 400X.

5- liver:

The changes in the liver tissue of rabbits, such as infiltration of lymphocytes and even necrosis, as a kind of tissue protection for itself by losing some of its cells to maintain the overall tissue and this is called apoptosis.

These results are in agreement with (Popper & Kent, 1975), which has shown an increase in the number of Kupffer phagocytes for some liver diseases as an active defense mechanism against harmful agents. The occurrence of cellular degeneration followed by necrosis, especially after increasing the concentration of sodium benzoate in the second group N2 in the current study.

(Elwi et al. 1973) explain necrosis as the local death of group tissues after a severe breakdown. They add that the early changes in the dead cells were swelling of the cytoplasm due to fluid imbibition within the cell and clotting of epithelial cells in the protoplasm.

After that, cells lose their membranes and become indistinct from one another. The shrunken nucleus and the chromatin became dense and dark, the nuclear membrane was torn and the nucleus was broken into small pieces; "karyorhexis". On the other hand, (El-Banhawy & Ganzuri, 1980) attributed this result to the harmful effects of many drugs on lysosomal membranes as they lead to the rupture of these membranes, which are very sensitive to any disease effect, and then release their strong enzymes, which cause degeneration And dissolve the various cellular components.

The congestion of the blood vessels can be attributed to the inflammatory response by the animal body to the harmful effects of toxic substances, which was characterized by increased blood flow to the area of injury. It may also be the result of damage and destruction of the lining of blood vessels by the action of toxic substances or oxidizing materials, as damage and destruction may occur in the endothelial lining of the blood vessels.

The current study revealed that the cytoplasm of hepatocytes treated with sodium benzoate revealed an increase in the amount of hepatic cellular lipid deposits. These results are

consistent with what was reported by (Khattab) (2007) of a similar result in mice after ingesting sodium benzoate.

As for necrosis and degeneration in hepatocytes and their enlargement, the reason may be attributed to the amount of sodium present in the benzoate substance, which in turn affects the normal proportions present in the cells and this is consistent with the results of a study conducted by (Sinha & D'souza, 2010) in Mice after sodium benzoate treatment, these degenerative changes may be due to the removal of sodium from the cells following decreased ATP supply. Thus, reducing the energy required to regulate cell ion concentration may play a role in the distention and swelling of hepatocytes. This indicates the energy requirements of the cell in an effort to overcome toxicity (Elwi, 1967).

Scheffel (1988) suggested that exposure to certain toxic substances affects ribosomes and their ability to produce peptide chains. As a result of the reduction in peptide synthesis, the amount of proteins involved in the transport of triglycerides decreases, and hepatocytes and tough proteins such as lipid-metabolizing enzymes are also lost (Holm et al., 1993). At the same time, triglycerides are produced continuously at a normal rate, which leads to Gradual accumulation of fat globules.

In the current research, the diversity of the cytoplasm of hepatocytes could be due to degradation of the cell organelle and especially the mitochondria with a subsequent decrease in the sodium and potassium pumps followed by the accumulation of water. These results are in agreement with (Fujitani, 1993), where swelling of some hepatocytes was observed by sodium benzoate.

The current study also revealed an increase in the number of Kupffer cells in groups treated with sodium benzoate. The results are consistent with the findings of (El-Shamy et al 1999) in a study conducted on a rat after being fed a supplementary diet.

In the current research, a remarkable accumulation of collagen fibers was detected in the livers of rabbits treated with sodium benzoate, and fibrosis can be classified as a wound response - healing for a variety of chronic stimuli resulting from chronic infection due to various causes (viral, toxic, metabolism, autoimmune), Which ultimately leads to cirrhosis.

(Beljaars et al. 2002) indicated that the center of hepatic fibrosis is the hepatic stellate cell (HSC).

(Greff) (2007), indicates that sodium benzoate has the potential to cause severe DNA damage in mitochondria to the point that it completely disrupts DNA. Greff (2007) applied the incidence of abnormalities of fetal organs in the eyes, brain, and kidneys.(Khaleel,et al 2019) (Park.et al, 2011) who observed the harmful effects of sodium benzoate on vital parts of mitochondrial DNA, and that sodium benzoate can cause many diseases including Parkinson's and other nerves.

6- The kidneys

The appearance of swelling in the tubules as well as the swelling of the nuclei Auxes of Nucleus is an indication of the cells being affected by high doses of sodium benzoate. 2014)) Injury of the nuclei and the basement membrane of the tubular cells and the breakdown of the glomerular structure, and the injury of the visceral epithelium in the renal tissue by microscopy.

From the study of the kidneys, it was concluded that it is an organ highly susceptible to reactive oxygen species (ROS), most likely due to the abundance of long-chain polyunsaturated fatty acids.

From their study, it was shown that the kidney damage was greater than the liver damage in rabbits treated with sodium benzoate due to the significantly increased level of lipids in the kidneys. The level of the liver's antioxidant defense system that prevents damage to these tissues.

Results of catalase activity in kidney tissues showed that all doses of sodium benzoate significantly reduced its activity compared to the control group. This is the same results of the previous study on the effect of sodium benzoate on catalase activity in erythrocytes (Yetuk, 2014). They suggested that the reduction in catalase enzyme and glutathione peroxidase and activities in erythrocytes by sodium benzoate may be due to damage in these antioxidant enzymes by ions. Ultra-oxidative stress, it has also been found that enzymes and other detoxification systems such as thioredoxin and GSH peroxidase have important roles in causing oxidative stress and reductase in mammals (Jones, et al, 1981) (Turanov, et al, 2010).

A study conducted by (Oyewolee et al.,2018) (Khoshnoud et al. 2012) with a two-week treatment of 200 mg / kg sodium benzoate oral dose caused some harmful effects on liver and kidney function in mice. Different organ tissues have different antioxidant capacity to deal with oxidative stress, and in the mouse that was using sodium benzoate, treatment with benzoate showed Sodium deficiency in behavioral tests, such as learning and memory.

The overall changes in the tissues of the organs can be attributed to the effect of benzoates by causing oxidative stress to cells, as benzoates lead to the generation of free radicals by oxidizing fats and other organelles, especially their membranes, as well as affecting structural proteins in the cell, leading to the stimulation of programmed death known as Apoptosis, all of which negatively affects body tissues, including the liver and kidneys.

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