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# *Response to a Popular Preservative Following Exposure to Sunlight during Storage*

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## *Abstract*

The purpose of this study was to determine the effects of sodium benzoate (SB) and apple juice containing SB on male rats after storage for 2 and 4 months in the sunshine. High-performance liquid chromatography (HPLC) was used to determine the concentration of sodium benzoate (SB) in the apple juice during each of the three storage stages (starting time, after 2 & 4 months). Ninety-nine white male rats were divided into three stages (starting time, after 2 & 4 months), each stage containing 3 groups (11 rats each): control group, aqueous SB solution, and apple juice containing SB. Biochemical factors "kidney functions," biomarker oxidation in brain tissues, such as glutathione (GSH) and malondialdehyde (MDA), as well as the brain and kidney histological examination, were evaluated / stage. Other biological factors included feed intake (FI) and the weight gain of the rats (BWG). The findings showed that at the three storage stages, the G2 rats' FI and BWG levels were considerably higher than those of the healthy rats' G1 levels. Throughout the many

stages, G2 performs the worst. Over the whole period, the healthy group had the highest GSH and the lowest MDA values. Both groups of SB (chemical solution SB and apple juice containing SB) damaged the rats' kidneys and brains. This damage was caused by the oxidation situation.

**Keywords**: Preservatives, Sodium Benzoate, Rats Brain

## *Introduction*

The issue of using food preservatives has received considerable critical attention. In the last years, with the excessive production and consumption of processed and convenience foods, the use of food additives has increased enormously ***(Wang et al., 2016).***Preservatives make up the majority of these additives, which are broadly utilized for a variety of purposes ***(Esimbekova et al., 2017).*** Food preservatives are additives used to stop food from deteriorating as a result of oxygen exposure, enzymes, and microbes ***(MacDonald and Reitmeier, 2017).***Sodium benzoate (SB) is one of the common food preservatives***(Kehinde et al., 2018),*** known sodium salt of benzoic acid with the chemical formula $C_7H_5NaO_2$ and number E211 ***(Moeen et al., 2018).*** Its strong solubility in water and good stability has led to its widespread use in a variety of food products, including margarine, sauce, marmalade, gelatin, liqueurs, beer, fruit juice, and soft drinks ***(Bruna et al., 2018).***

***Asejeje et al., (2022)*** reported that SB is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA, 1991), with a 0.1% (1000 ppm) permissible limit level in food. Additionally, at dosages of 647-825 mg/kg of body weight per day, the International Program on Chemical Safety (IPCS) revealed no

significant health effects in humans ***(Noorafshan et al., 2014).*** However, numerous types of research indicate that consuming SB causes DNA damage, urticaria, angioedema, asthma, childhood hyperactivity, and anxiety ***(Gaur et al, 2018).***

***Pizzorno (2015)*** stated that through tubular concentration and excretion into the urine, the kidneys play a specific role in the body's removal of harmful substances. These capabilities make it vulnerable to harm from repeated exposure to hazardous metabolites or chemicals ***(Kataria et al., 2015).*** The kidneys remove SB further, and continued use of it may have adverse effects similar to those caused by toxins as ***Lennerz et al., (2015)*** declared.So, the aim of this study was conducted to evaluate the effect of storage exposure of sodium benzoate to sunlight and its effect on the brain and kidneys of rats.

## *Materials and Methods*

### Materials

The chemicals and kits were obtained From El-Gomhorya Company for chemicals and drugs (Cairo, Egypt). Juice apples preserved with SB and corn oil required for preparing experimental diets were bought from local-market, in Cairo, Egypt. Animals used in experiments were bought from the Cairo, Egypt-based vaccine and immunity organization Helwan Farm, 99 healthy adult male white rats of the "Sprague Dawley strain" (150 gm±10) were purchased for the experiment. In agreement with ***Reeves (1993)***a standard diet was formulated.

### Methods

**Storage of Juice:**

Two and four months of solar exposure were given to apple juice in transparent glass bottles containing SB.

Determination of SB in apple juice at starting time and after two, to four months of storage in sunlight using HPLC, was done according to ***Astuti et al., (2019).***In that study, the determination of SB levels in apple juice drinks was performed using an HPLC method using a Photodiode-Array Detector (PAD). Methanol pro-HPLC: aqua pro admission (70:30) made up the mobile phase, and glacial acetic acid was added to it to bring the pH level down to 3. The analysis was carried out at a 245 nm wavelength with a flow rate of 1 ml/min.

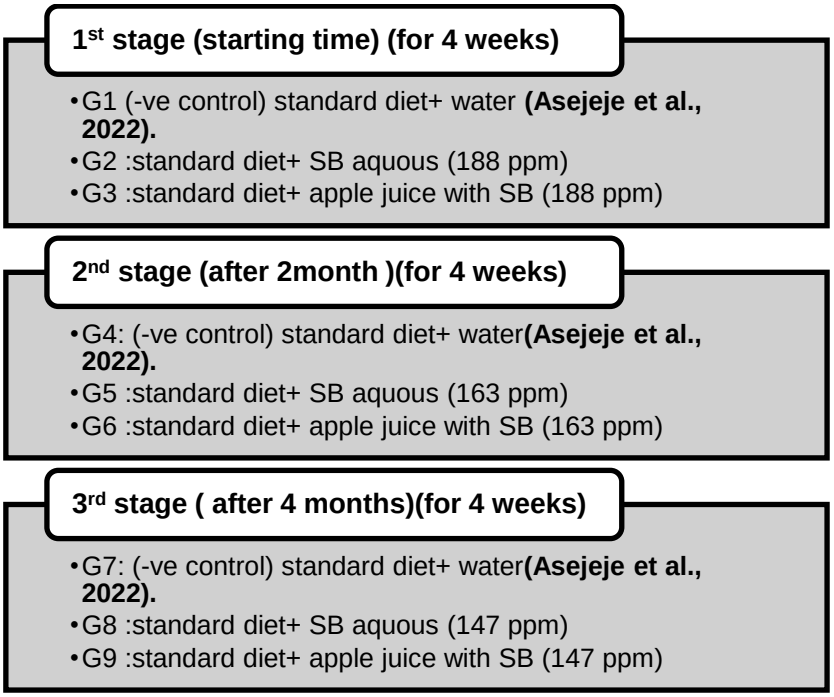
**Biological experiment**

In clean, single-wire cages with wire bottoms, 99 healthy adult male white rats of the "Sprague Dawley strain" were kept (11rat/group). The rats were given meals in unique feeding containers to prevent food spillage. The rats were also given water via a glass tube that was projected through their wire cages. Ad-libitum supplies of food and water were offered and monitored twice a week.

**Experimental design**

The current study was approved by the National Hepatology & Tropical Medicine Research Institute (NHTMRI) (NO: A9-2022).

**7 days for adaptation then rats were divided into3 stages, each stage containing 3 groups (11rats/group) as follows:**




The animals were sacrificed under anesthesia at the end of each stage of the experiment (4 weeks), and blood samples were obtained using retro-orbital injection and placed in dry centrifuge tubes. Additionally, the kidney and brain were collected. A portion of them was placed in 10% formaldehyde to examine the histology, and another section was placed at - 20°C to identify oxidation biomarkers. Until analysis, serum was stored in plastic vials at - 20 °C.

**Biological Assessment**

**Feed intake (FI) and growth rate calculations**

To calculate daily feed intake (FI; in grams), the amount of food left in the cage was subtracted from the amount of food given to each animal each day ***(Manjula and Krishna, 2016).*** Weekly measurements were taken of the rats' overall growth rates throughout the trial, and the rates of growth for each group were calculated as follows:

Growth can be defined as the difference between current weekly weights (g) and previous weekly weights (g) **(Adeyemi et al., 2015).**

**Biochemical *Evaluations***

The method of***Young, (2001)***was used to measure serum creatinine, urea, and uric acid.

**Identification of antioxidant and oxidative biomarkers**

Malondialdehyde (MDA) is detected in tissue analysis by ***Botsoglou et al., (1994).***While the spectro-photometric technique was used to measure glutathione (GSH) according to ***Goldberg et al., (1983).***

**Determination of Relative organs (brain& kidney) Weight:**

Following the sacrifice of animals, carefully dissecting and weighing the kidney and brain (absolute weight).***Al-Attar, (2010)***stated that "Relative Organ Weight = [Absolute Organ Weight (g) / Final Body Weight of Rat (g)] ×100"

**Statistic evaluation**

The information was presented as mean ± SE (standard error). To evaluate the research hypothesis, a variance analysis (ANOVA) was conducted, followed by a posthoc least significant difference (LSD) test. Utilizing the Science Statistical Package (SPSS) version, data analyses were carried out. Statistical significance was defined as a two-tailed *P* value < 0.05***(Daniel, 2005).***

## *Results and Discussion*

**Chemical Evaluation of sodium benzoate (SB)**

Fig (1) explained the determination of SB in apple juice by HPLC through the different storage times (starting time, after two and

four months). According to the findings in Fig (1), the highest SB concentration was found at the beginning (188 ppm), and it decreased by 13% after two months of exposure to sunlight.

The lowest value was found in the juice after four months (147 ppm) suggesting that some of the SB may have been lost to benzene conversion. According to **Salviano dos *Santos et al., (2015),*** the breakdown of SB was a source of benzene in foods and a human carcinogen. The juice's shelf life may be impacted by this loss of SB. The production of benzene from SB breakdown in the presence of vitamin C was studied by ***Mcneal et al., (1993).***

They used aqueous models containing vitamin C and SB to assess how temperature and UV exposure affected the production of benzene. Who discovered that "300 ng" benzene was produced in 20 hours of UV or 45°C storage in 1 ml of water.Additionally, ***Aprea et al., (2008)*** investigated how the quantities of conformable vitamin C and SB in processed drinks affected the generation of benzene concerning the amount of heat present in the solution. Benzene production in the aqueous solution held at 25°C stayed constant for the first 12 hours (0.01 ppb) but increased to 0.44 ppb by the end of the 72-hour experiment. In the model solution held at 45°C, benzene developed in the following 24 hours and reached 125 ppb in the next 48 hours.

**Effects of sodium benzoate on biological processes**

The findings in Fig (2) demonstrated how the administration of sodium benzoate (SB) during storage periods affected feed intake (FI) and body weight gain (BWG) in various experimental groups. A one-way ANOVA analysis revealed that the difference in FI& BWG values between the experimental groups at the beginning was non-significant (G1, G2, and G3). Comparing the SB groups (G2, G3, G5, G6, G8, G9) to the control group (G1, G4, G7), the highest FI& BWG

values were seen. These findings concur with those of ***Olofinnade et al., (2021 a).***

***Yilmaz and Karabay (2018)*** reported that the immunological modulatory activity of SB has inhibited leptin hormone release with an adiposity model in vitro, highlighting the importance of SB to the development of obesity ***(Piper and Piper, 2017).*** Apple juice groups (apple juice with SB) in the overall experiment (G3, G6, and G9) had the greatest FI and BWG Fig (2a, 2b). This is probably a result of the sugar in apple juice. When compared to other SB concentrations, the results in Fig ($2^a$, $2^b$) showed that FI and BWG values were significantly higher ($P < 0.0001$) at concentration 147ppm (3rd stage), which is the lowest SB concentration than (188ppm, 163ppm).

These findings corroborated ***Olofinnade et al. (2021 b)*** observation that an increase in FI at 125 mg/kg (the lowest dose) is associated with a rise in weight, indicating that SB may boost food palatability at this concentration. At larger doses, FI and BWG decreased, most likely as a result of how SB affects the taste of food. An earlier study that showed that continuous exposure to SB in mice was linked to lower BWG and lower growth supports this finding ***(Griffith, 1929; Nair, 2001).*** The decrease in BWG seen with SB has also been linked to a lack of blood phospholipids and nutritional components like glycine ***(Griffith, 1929; Kowalewski, 1960).***

**The brain's reaction to Sodium Benzoate (SB)**

In maintaining cellular redox homeostasis, the involvement of the antioxidant defense mechanisms involving endogenous reduced glutathione and the enzymatic antioxidants in alleviating oxidative stress are crucial ***(Łuczaj et al., 2017).***

The purpose of this study was to examine the impact of SB on MDA and GSH levels, which serve as antioxidant indicators in the rat brain, based on the studies about the toxic effects of SB that were previously mentioned and the widespread use of this food additive in beverages, medications, and cosmetics products.Fig (3) indicated the Effect of Sodium Benzoate (SB) on oxidative brain state markers (GSH& MDA) and Brain relative weight (RW) values in different experimental groups.From Fig (3a, 3b) during 1st stage of storage, there was no significant between MDA& GSH of experimental groups (G1, G2& G3). While the results in the $2^{nd}$&$3^{rd}$ stages (G5, G6, G8, and G9) showed a highly significant elevation of brain oxidant activities (MDA) and the reduction of GSH levels ($P < 0.0001$).

Within the 1st stage (starting time; 188ppm SB), RW of G2 highly significant decreased ($P < 0.0001$; decreased by 24%) compared to control group (G1) but moderately significant decreased ($P < 0.01$) in SB juice group (G3; decreased by 18%) as showed in fig. (3c)

From Fig (3c), there was no significant difference in RW of G5 whereas RW of G8 highly significant levels ($P < 0.0001$) diminished compared with the SB group with the highest SB concentration (G2).

The results showed that SB caused histological alterations in brain tissue. Similar to G2, meningeal blood vessel congestion and perivascular cuffing with inflammatory cells were seen at the first storage stage (Fig 4b), whereas brain tissue from control-positive rats (G5) displayed extensive neuronal cell degenerative changes, including vacuolar degeneration, necrosis, nuclear pyknosis, and neuronophagia (Fig 4e). With modest vacuolation of the neuropil, it was possible to see neuronal cell vacuolation, necrosis, and apoptosis as well as perivascular edema. Some dead neurons looked like ghost cells (G8; Fig 4h). Additionally, according to Fig. 3c, the RW of SB (G8) and apple juice containing SB (G9) decreased by

49% and 35%, respectively, during the third stage (after four months of storage), compared to the healthy control group at this period (G7). G6 demonstrated a good restoration degree of the cerebral cortical neuronal cells with mild necrobiotic changes (Fig 4f); While G9 demonstrated good protection of the cerebral neurons with only a mild degree of degenerative changes and few necrotic ones as explained in Fig (4i). G3 also demonstrated normal histological structure. SB dramatically lowered GSH and elevated MDA levels in the brain, according to ***Khoshnoud et al., (2018).***

In the striatum, hippocampus, frontal cortex, and cerebellum of exposed rats, ***Asejeje et al., (2022)*** found that SB caused neurobehavioral damage and brain biochemical alterations via processes involving reduction of oxidative stress, TNF, and caspase-3 activation. GSH depletion, a critical Central Nervous System (CNS) antioxidant associated with cognitive decline and issues with short-term spatial memory, has been related to increased oxidative stress ***(Lin et al., 2021).*** Additionally, as previously established by researchers, different organ tissues have unique antioxidant capacities to respond to oxidative stress, as demonstrated by the decrease in GSH content and an increase in MDA in the rat brain after SB treatment. Additionally, behavioral testing revealed that SB therapy resulted in deficits in memory and cognition ***(Khoshnoud et al., 2018).***

As a D-amino acids oxidase inhibitor, SB has positive neuroprotective effects in pathological cases, but chronic consumption of SB in a healthy state is likely to be neurotoxic. According to previous research, activating the N-methyl-D-aspartate receptor (NMDAR) causes oxidative stress, which breaks down cellular macromolecules like lipids, DNA, and proteins ***(Seillier et al., 2022).***In male rats, the effects of sodium benzoate on oxidative stress have also been studied at various doses and for different periods of

time ***(Sabour and Ibrahim, 2019).*** Reduced levels of GSH and MDA were seen in several of the research groups. An increase in oxidative damage linked to benzoate's impact on oxidative stress. Another investigation verified this result ***(Olofinnade et al., 2021 b).*** In a study by ***Khan et al. (2022),*** SB was given to rats for 30 days at various doses (70, 200, 400, and 700 mg/kg body weight). The amount of 70 mg/kg used in this investigation was shown to be safe; however, at higher levels, the chemical lowered the activity of the antioxidant enzymes.

**Renal Impact of SB**

**Kidney Functions**

***Pizzorno (2015)*** explained that by concentrating toxic compounds in tubules and excreting them in urine, the kidneys play a crucial part in eliminating them from the body. These tasks make it susceptible to damage from long-term exposure to dangerous metabolites and toxins ***(Kataria et al., 2015).*** Then, like any other chemical, SB is eliminated by the kidneys, and chronic use may have detrimental effects ***(Piper and Piper, 2017).***

According to the results of the concentrations of uric acid, urea, and creatinine in serum shown in Fig (5), there are highly significant differences between the rats treated with SB and the negative control group in all examined biochemical parameters at level 0.0001.On the other hand, throughout the storage periods, the concentration of urea and creatinine also showed a significant increase ($P < 0.0001$) in G3, G6, and G9 compared to the control, with the rise being more prominent in the G2 group compared to the G1 group (Figure 5). (Starting time, after 2 and 4 months).

Our findings, which concur with ***Tawfek et al. (2015);Helal et al. (2019)***demonstrated a significantly significant increase in urea and creatinine levels. This may suggest that SB can affect kidney function, possibly as a result of the metabolites these food additives

have on the tissues of the kidneys. Additionally, serum levels of urea and creatinine elevated as kidney function in filtering bodily fluids declined. However, sodium benzoate might have disrupted the metabolism of creatinine, increasing its synthesis, or the tissues might have damaged its functional capacity for tubular excretion in whole or in part ***(Tawfik and Al-Badr, 2012).***

According to ***Radwan et al., (2020),*** SB (200 mg/kg) significantly raised the waste products urea and creatinine, which may have been a result of the enhanced peroxidation that SB caused, which led to kidney damage in SB-treated subjects. Blood urea and creatinine levels rise as a result of the kidneys' reduced excretory function caused by SB ***(Suljevi et al., 2022).***

The drop in urea and creatinine can attribute to the action of SB on serum glycine levels, which affects the levels of metabolites like urea and creatinine. Sodium benzoate is removed from the body by coupling with glycine, and high sodium concentrations cause more glycine to be lost by coupling. Reduced blood glycine thus impacts how quickly urea and creatinine are released. Lower glycine levels are known to reduce the generation of urea and creatinine in the urine, which causes these compounds to accumulate and their blood levels to rise***Oyewole et al., (2012).***

**Biomarker Lactate Dehydrogenase (LDH) Effect of Renal Toxicity**

In the human body, lactate dehydrogenase (LDH) is crucial for the anaerobic conversion of pyruvate to lactate and vice versa. In clinical practice, lactate levels are frequently used to assess the severity of an illness and the effectiveness of treatment interventions ***(Farhana and Lappin, 2022).*** All significant organs include the cytoplasmatic enzyme LDH. LDH has been utilized to identify cell

injury based on its extracellular appearance ***(Jaiswal et al., 2018).*** When cells or tissues are injured, LDH, which normally resides in the cells, is released into the media. Cell membrane integrity is compromised when there is a degree of LDH leakage ***(He et al., 2014).*** When comparing the rats treated with the chemical SB (G2,G5, G8) and the apple juice containing SB (G3, G6, G9) to the control group across the various storage times( G1, G4, G7), the concentration of LDH in serum showed the presence of significant differences at level $P < 0.05$. As a result, taking SB together with food may have an impact on how the body works and how it burns calories, raising LDH levels. These findings were consistent with those of ***Zeghib and Boutlelis (2021),*** who found that administering SB to rats induced a considerable increase in creatinine, urea, renal MDA levels, and LDH activity. The activity of AST and LDH in rats was shown to be enhanced by sodium benzoate ***(Ibekwe et al., 2007; RasGele and KaymaK, 2013).*** These findings supported those of ***Naik et al., (2021),*** who showed that benzoate administration led to appreciable modifications in LDH activity in the rat liver, kidney, testis, heart, cerebral cortex, cerebellum, and muscle.

**The relative weight of the kidney and its histopathology**

Fig (7) showed the impact of sodium benzoate (SB) on the relative weight of the kidneys (RW) in several experimental groups. The RW of G8 exhibited a highly significant rise ($P < 0.0001$), while that of G9 significantly increased ($P < 0.05$) compared to the control group, making the lowest SB concentration (147 ppm; 3rd stage) the most effective. Kidney tissue from the sodium benzoate group underwent histopathological evaluation, which demonstrated nephrotoxicity andsupported the biochemical findings.

Histopathological sections obtained from rat's kidney (Fig 8) showed the effect of SB in the structure of urinary tubules and renal glomeruli. Compared with the control group, this normally appeared

(Fig 8 a, d, g), while the section obtained from rats treated with SB revealed histological changes including renal tubular epithelial degeneration, some desquamation, and necrosis with the presence of granular and eosinophilic cast in the tubular lumen as well as thickening of the parietal layer of Bowman's capsule at 1st stage (as shown in Fig 8b), with kidney RW 0.6±0.06. Also, diffuse vacuolar degeneration, nuclear pyknosis and necrosis of the renal tubular epithelial linings, cell debris in the tubular lumen with shrinkage of the glomerular tuft (Fig. 7e and 7h), in addition to a good degree of restoration of the renal tissue, with a mild degree of tubular epithelia linings degenerative and necrotic changes with some desquamated cells and few congested interstitial blood vessels, especially in G3 (Fig 7c) with very few mononuclear inflammatory cells infiltrating the interstitial tissue (Fig. 7h) while at 3rd stage kidney showed a good degree of restoration of the renal tissue with mild necrobiotic changes of the tubular epithelial linings(Fig 7i). The absolute weight of the rats' kidneys increased as a result of sodium benzoate. Histological alterations were seen, the same as in the earlier research***(Helal and partner, 2019; Radwan et al., 2020),***which also supported the adverse effects on the kidney. The current investigation supports the findings of earlier studies.

According to ***Zeghib and Boutlelis (2021),*** a microscopic examination of the SB section showed severe glomerular and tubular change, glomeruli atrophy, nephrocellular necrosis, vascular congestion, inflammatory cell infiltration, vacuolization of tubular cells, and tubular dilatation. Additionally, cellular damage brought on by oxidative stress, which might happen as a result of SB injection, maybe the cause of pathohistological alterations ***(El Hassani et al., 2019).***

In addition to having an inflammatory effect on the renal tissue, sodium benzoate has been shown by ***Hasson et al. (2021)*** to have a detrimental impact on renal efficiency via decreasing erythropoiesis. It also resulted in renal tubule disorganization and disruption of urea, creatinine, and balance control and balance, all of which pose a threat to the development of chronic kidney disease and its consequences, such as cardiovascular illnesses.

## *Conclusion and Recommendation*

When antioxidant enzymes are ineffective, sodium benzoate creates oxidative stress, harming inside tissues (kidney and brain). A rise in the ultimate body weight in the $2^{nd}$ and $3^{rd}$ stages, and an excess of renal functions (creatinine, urea, and uric acid) all indicated that there had been tissue damage in this study. We recommended that: Utilizing natural preservatives in place of synthetic ones, reducing adult intake and use of artificial preservatives while outlawing their use in children's foods, and avoiding storing juices in direct sunlight, provided that they are packed in opaque glass bottles.

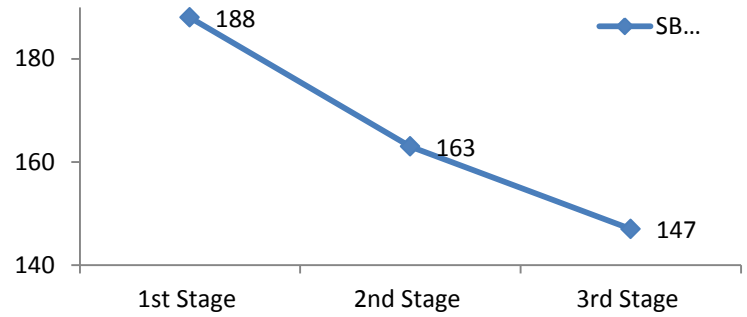



**Fig (1):**

Determination of SB ( sodium benzoate) in apple juice by HPLC; LOQ =25 mg/Kg, uncertainty is ± 34% of the test methods 1st Stage: starting time, 2nd stage:2 months of storage, 3rd stage: 4 months of storage

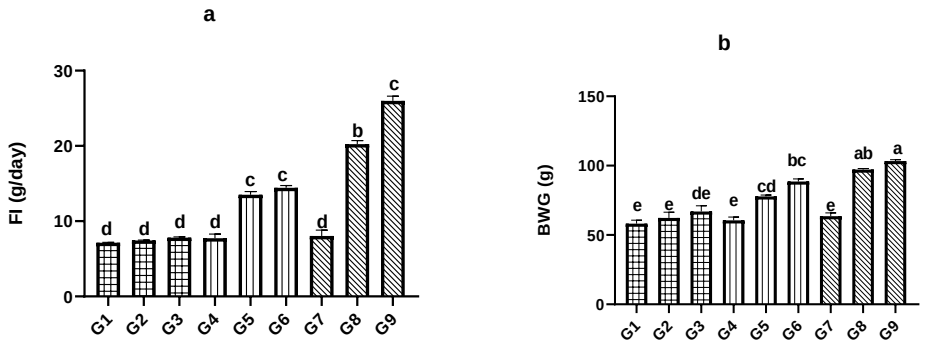



**Figure (2):**

Effect of Sodium Benzoate (SB) on a) feed intake (FI), b) body weight gain (BWG) value in different experimental groups during storage periods. (a-e) Represents the mean value ± S.E. (n=11 rats/group), Means that do not share a letter are significantly different using One-way ANOVA ($P < 0.05$)

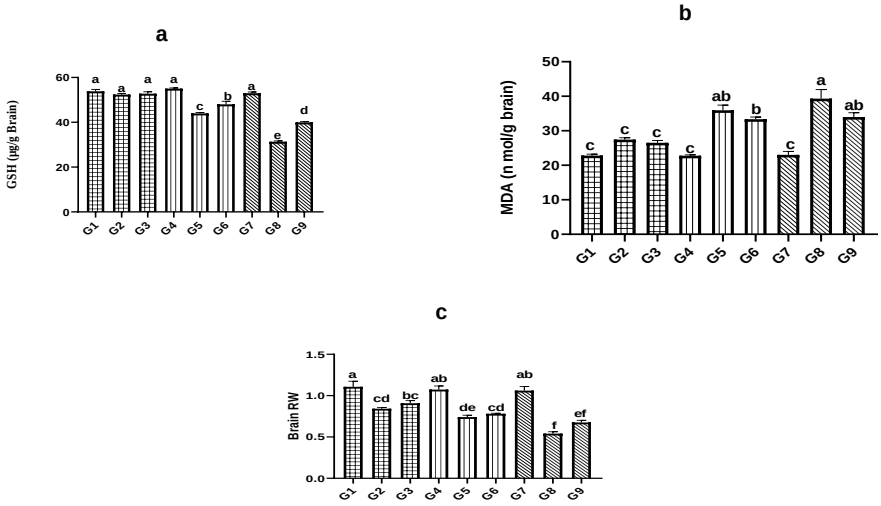



**Figure (3):**

Effect of Sodium Benzoate (SB) on oxidative brain state markers & RW: a) GSH, b) MDA& c) Brain relative weight (RW) value in different experimental groups. (a-e) Represents the mean value ± S.E. (n=11 rats/group), Means that do not share a letter are significantly different using One-way ANOVA ($P < 0.05$)

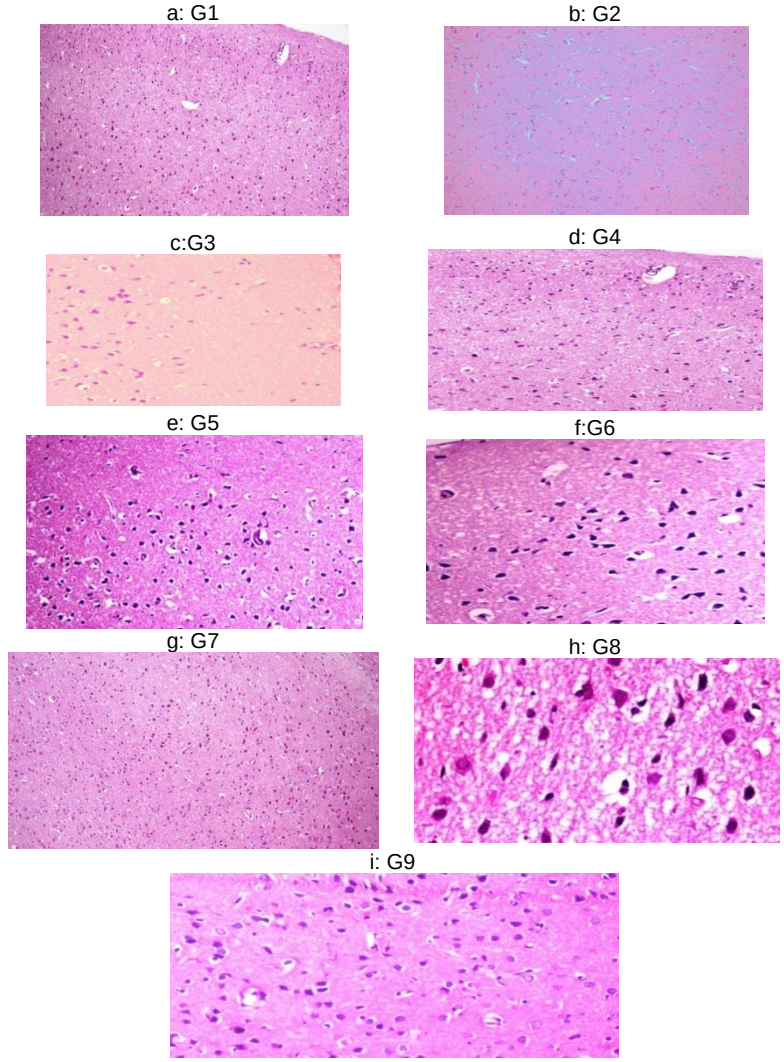



**Fig. (4).**
TS of Brain of a) G1: Normal control group showed the normal histological structureb) G2: shows perivascular cuffing with inflammatory cells, c) G3: shows the normal histological structure, d) G4: Normal control group showed normal histological structure, e) G5: showed widespread neuronal cell degenerative changes, particularly vacuolar degeneration, necrosis, nuclear pyknosis, and neuronophagia, f) G6: good degree of restoration of the cerebral cortical neuronal cells with mild necrobiotic changes, g) G7: Normal control group showed, h)G8: good degree of restoration of the cerebral cortical neuronal cells with mild necrobiotic changes, i) G9 showed good protection of the cerebral neurons with only mild degree of degenerative changes and few necrotic ones.

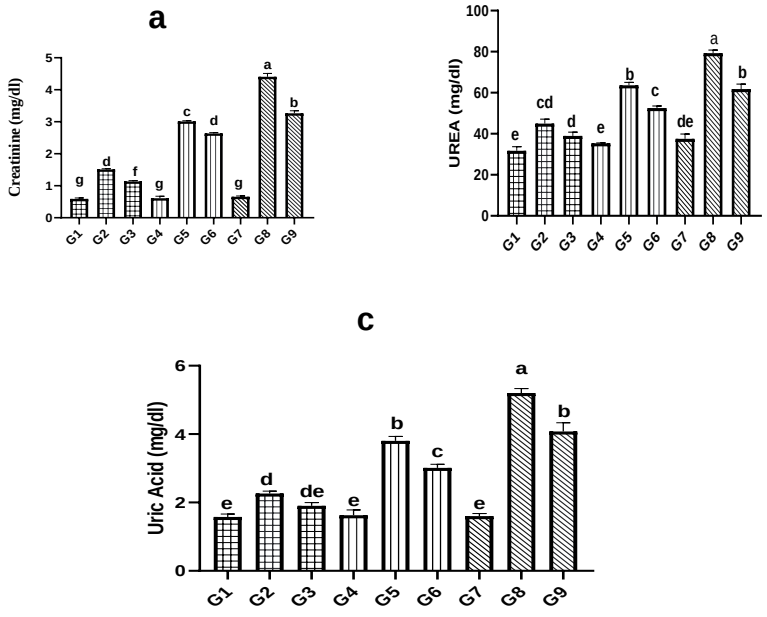



**Figure (5):**
Effect of Sodium Benzoate (SB) on Kidney functions: a) Creatinine, b) Urea& c) Uric Acid value in different experimental groups.
(a-e) Represents the mean value ± S.E. (n=11 rats/group), Means that do not share a letter are significantly different using One-way ANOVA ($P < 0.05$)

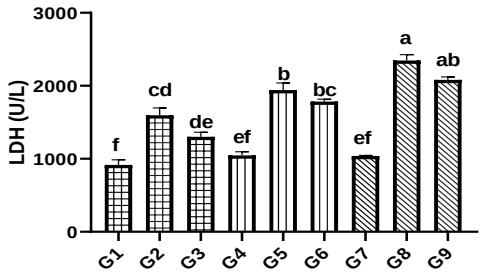



**Figure (6):**
Effect of Sodium Benzoate (SB) on lactate dehydrogenase (LDH) in different experimental groups.
(a-e) Represents the mean value ± S.E. (n=11 rats/group), Means that do not share a letter are significantly different using One-way ANOVA ($P < 0.05$)

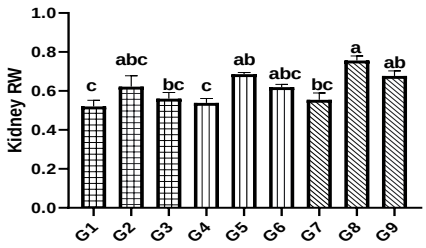



**Figure (7):**

Effect of Sodium Benzoate (SB) on kidney relative weight (RW) in different experimental groups. (a-e) Represents the mean value ± S.E. (n=11 rats / group), Means that do not share a letter are significantly different using One-way ANOVA ($P < 0.05$)

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# استجابة الجرذان لبعض المواد حافظة شائعة الاستخدام بعد التعرض لأشعة الشمس أثناء التخزين

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## الملخص العربى

كان الغرض من هذه الدراسة هو تحديد تأثير بنزوات الصوديوم وعصير التفاح المحتوي عليها على ذكور الجرذان عند بداية التجربة و بعد التخزين لمدة شهرين و 4 أشهر في ضوء الشمس. و قد تم استخدام الكروماتوجرافيا السائلة عالية الأداء لتحديد تركيز بنزوات الصوديوم في عصير التفاح خلال كل مرحلة من مراحل التخزين الثلاث (وقت بداية التجربة و بعد التخزين لمدة شهرين و 4 أشهر) . ثم تم تقسيم تسعة وتسعين جرذ ذكور بيضاء إلى ثلاث مستويات (وقت بداية التجربة و بعد التخزين لمدة شهرين و 4 أشهر) كل مستوى يحتوى على ثلاث مجموعات (11 جرذًا لكل منها): مجموعة التحكم , محلول المائى لبنزوات الصوديوم وعصير التفاح المحتوي على بنزوات الصوديوم. و قد تم تقييم بعض العوامل البيوكيميائية "وظائف الكلى" ، والعلامات الحيويةللأكسدة في أنسجة المخ ، مثل الجلوتاثيون و MDA ، وكذلك الفحص النسيجي للمخ والكلى. وشملت العوامل البيولوجية الأخرى تناول الغذاء وزيادة وزن الجرذان. أظهرت النتائج أنه في مراحل التخزين الثلاث ، كانت مستويات تناول الغذاء وزيادة الوزن للجرذان للمجموعة الثانية أعلى بكثير من مستويات مجموعة الجرذان الصحية. خلال العديد من المراحل ، كان أداء المجموعة الثانية هو الأسوء على مدار الفترة بأكملها ، كان لدى المجموعة السليمة مستوى للجلوتاثيون كان اعلى بينما كانت قيم MDA أقل فى كلتا المجموعتين من صوديوم بنزوات (سواء المحلول كيميائي اوالعصير) تسببا في تلف الكلى والمخ لدى الجرذان. و يعزى ذلك الضرر بسبب حالة الأكسدة.

**الكلمات المفتاحية**: المواد الحافظة – بنزوات الصديوم -الجرذان