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Preservative Sodium Benzoate as a Food Additive Alone and in Combination with Vitamin C and its Toxic Effects on the Liver and Kidney Function of Adult Male Rabbits

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Abstract

The present study was designed to determine the adverse effect of sodium benzoate (SB), vitamin C (Vit.C) alone and their combination on liver and kidney functions in rabbits. Thirty-six adult male rabbits were divided randomly into six equal groups. Group 1 (Control group) received orally distal water, group 2: Vit.C (200mg/kg BW/day) received orally, group 3: received orally SB (60 mg/kg BW/day), group 4: received orally SB (120 mg/kg BW/day). Group 5: received orally SB+Vit.C (60+200 mg/ kg BW/day) and group 6: received orally SB+Vit.C (120+200 mg/ kg BW/day). The result revealed a significant increment in ALT, AST, ALP and MDA in groups 4, 5 and 6 while total protein levels increased in G3 and G4 and decrease in G5 and G6 compared to control and another treated groups. The urea and creatinine levels increased in G5 and G6 as compared to another groups. In conclusion that SB administration alone and together with Vit.C caused adverse effect on liver and kidney functions compared to the group treated with the Vit.C alone and control groups.

Keywords: Sodium benzoate, Vitamin C, combination, liver, kidney.

Introduction

Sodium benzoate (SB) occurs naturally along with benzoic acid and it esters in many foods fruits and vegetables can be rich sources particularly berries such as cranberry and bilberry, other sources include seafood such as prawns, and dairy product like milk, cheese and yogurt (1). Sodium benzoate is used as preservatives in beverages, fruit products, chemically leavened baked goods, and condiments,

preferably in a pH range under 4-5. The disadvantage is the off-flavor they may important foods Owing to their inhibitory effect on yeast, they cannot be used in yeast-leavened products (2) The upper concentrations allowed in food are up to 0.1% benzoic acid between 0.15% and 0.25% (other countries) (3). The European Commission limits for benzoic acid and sodium benzoate are 0.015–0.5% (4). Benzoic acid and its salts and esters are found in 11 of 48 (23%) toothpastes (5) to a maximum of 0.5% (6) Sodium benzoate also used in cosmetics (7). Sodium benzoate is as a safe substance, but short-term exposure can cause irritation of eyes, skin and respiratory tract, yet prolonged or repeated contact may cause high skin sensitization chromatogram (2004) who was ulcers and gastric mucus secretion changes (8, 9). In a study conducted during year 2007 sodium benzoate increased blood pressure, eventually tearing the vessels in the rat (10). Damage to the hepatocyte cell membrane and crista losses in mitochondria, connection to outer shell of vacuole mitochondria in the cytoplasm and liver and kidney dysfunction are other adverse effects of consuming sodium benzoate (11).

Materials and Methods

Animals and Housing: Thirty-six adult male rabbits were used in this study. All rabbits were weighed about (205.00±19.00). They were kept in animal house under constant environmental condition for 2 weeks to acclimatize before that beginning of the experiment. Food and drinking water were provided *ad libitum* throughout the experiment.

Experimental Design: Adult male rabbits were divided randomly into six equal groups as follows: Group 1 (control): six adult male rabbits orally administered distilled water (4 ml/animal) by gavage daily for 30 days.

Group 2: Six adult male rabbits were orally administered Vit.C (200 mg/kg BW) (6) dissolved in 4 ml distilled water by gavage daily for 30 days.

Group 3: Six adult male rabbits were orally administered sodium benzoate (60 mg/Kg BW) (7) dissolved in 4 ml distilled water by gavage daily for 30 days.

Group 4: Six adult male rabbits were orally administered sodium benzoate (120 mg/Kg BW) (7) dissolved in 4 ml distilled water by gavage daily for 30 days.

Group 5: Six adult male rabbits were orally administered sodium benzoate (60 mg/Kg BW) combination with Vit.C (200 mg/kg BW) dissolved in 4 ml distilled water by gavage daily for 30 days.

Group 6: Six adult male rabbits were orally administered sodium benzoate (120 mg/Kg BW) combination with Vit.C (200 mg/kg BW) dissolved in 4 ml distilled water by gavage daily for 30 days.

Determination of Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) estimations (U/I): Aspartate and alanine aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (12).

Determination of Serum Alkaline Phosphatase (ALP) estimation (U/I): This estimation was done by using the colorimetric determination of alkaline

phosphatase activity (Biomeriexu, France) (13)

Determination of Serum Malondialdehyde measurements (MDA): The main end product of lipid peroxidation is Malondialdehyde, will be carried out in serum according to Yagi method (14). The base of this principle on the spectrophotometer measurement. Thiobarbituric acid (TBA) reacts with MDA to form thiobarbituric acid reactive substance.

Total protein measurement: Colorimetric method is described by (15, 16). The peptide bounds of protein react with Cu^{+2} in alkaline solution to form a colored complex which absorbance, proportional to the concentration of total protein in the specimen, is measured at 550 nm. The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution. The total protein was estimated by using a special chemical kit prepared by BIOLABO, SA, Maizy/ France).

Urea measurement: In this assay system the reaction involved is as follows:

The urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide (17). The serum urea concentration was measured by using a special chemical kit (SPECTRUM-Egyptian Company for Biotechnology).

Serum creatinine measurement: The particular feature of metabolism processes of muscle contraction is creatine and phosphocreatine; it is converted to a waste product creatinine. The amount of creatinine produced/day is related to a muscle mass, body weight, sex, age, diet and exercise. Creatinine is endogenously produced and released to body fluids at a stable rate and its plasma and serum levels are maintained within narrow limits, it can be measured as an indicator of glomerular filtration rate (GFR) (18). Calculate the results as follows:

Statistical Analysis: The data of results were expressed as mean ± standard deviation (M±SD), the experiments analyzed by using One-way ANOVA by SPSS (Special Program for Statistical System) version 21.0. The least significant difference test (LSD) was used to determine the differences between groups in ANOVA-test, the level significant set on ($p< 0.05$) (19).

Results

Effect of Sodium benzoate (SB) and Vit.C alone and their combination on serum liver enzymes and MDA in adult male rabbits: Table (1) showed a significant ($p<0.05$) increase of serum ALT in the G2, G3, G4, G5 and G6 as compared with the control group G1 and G2 (Vit.C). This table is also showed increase of AST in all the treated groups, but the more significant ($p< 0.05$) increase was in G5 (given SB 60 mg + Vit. C) and G6 (given SB 120 mg +Vit.C). It is also revealed a significant ($p< 0.05$) increase of ALP in G3, G4, G5 and G6 as compared with G2 (Vit. C) and G1 (control group). The more significant increase was in G5 and G6.Table (2) showed that there is significant ($p< 0.05$) decrease of serum protein in G5 and G6 compared with the control and other treated groups. The results also showed that there is significant ($p<0.05$) increase of serum urea concentration in all groups as compared with

the control and Vit.C, while it is more significantly ($p<0.05$) increased in group 5 and 6 (in which the combination of SB and Vit.C were given) as compared with the control group and Vit.C. The table also revealed significant ($p<0.05$) increase of serum creatinine in G5 and G6 more significantly ($p< 0.05$) than other treated groups and control groups.

Table (1) Effect of SB, and Vit.C alone and the ALT, AST, ALP and MDA levels in adult male rabbits (n=10):

Parameters Groups	ALT (U/l)	AST (U/l)	ALP (U/l)	MDA (U/l)
G1(control)	17.80 ± 0.28^{E}	15.50 ± 1.64^{E}	8.16 ± 0.75^{E}	5.58 ± 0.45^{D}
G2(Vit.C)	35.66 ± 1.63^{D}	29.00 ± 4.33^{D}	8.91 ± 0.80^{AE}	5.90 ± 0.61^{D}
G3 SB (60 mg)	46.00 ± 4.33^{C}	39.33 ± 1.21^{C}	9.66 ± 1.36^{A}	6.98 ± 0.52^{C}
G4 SB (120 mg)	81.16 ± 4.62^{B}	57.50 ± 7.28^{B}	11.16 ± 1.16^{B}	7.54 ± 0.27^{B}
G5(SB 60+Vit.C)	88.00 ± 4.73^{A}	74.83 ± 5.54^{A}	14.00 ± 0.89^{C}	8.39 ± 0.38^{A}
G6(SB 120+Vit.C)	86.00 ± 3.52^{A}	77.83 ± 7.38^{A}	18.66 ± 1.50^{D}	8.85 ± 0.19^{A}
LSD	4.83	10.33	1.50	0.56

Values expressed in capital letters mean significant differences at ($p< 0.05$) levels (M±SD).

Table (2) Effect of Sodium benzoate (SB) on serum Glu., total protein, urea and creatinine concentrations in adult male rabbits:

Parameters Groups	Total protein g/L	Urea mg/L	Creatinine mg/dl
G1(control)	9.92 ± 0.87^{A}	48.34 ± 2.28^{E}	1.09 ± 0.66^{D}
G2(Vit.C)	9.28 ± 2.24^{A}	61.62 ± 4.78^{D}	1.29 ± 0.11^{C}
G3(SB 60 mg)	10.53 ± 1.22^{A}	70.05 ± 5.25^{C}	1.38 ± 0.21^{C}
G4(SB 120 mg)	11.07 ± 1.22^{A}	84.45 ± 4.82^{B}	1.57 ± 0.18^{C}
G5(SB 60+Vit.C)	6.15 ± 0.76^{B}	88.64 ± 1.98^{B}	2.63 ± 0.32^{B}
G6(SB 120+Vit.C)	4.95 ± 0.40^{B}	101.61 ± 6.97^{A}	3.66 ± 0.09^{A}
LSD	1.78	8.43	0.27

Values expressed in capital letters mean significant differences at ($p< 0.05$) levels (M±SD).

Discussion:

The current study as illustrated in table (1), showed elevations of ALP, AST and ALT levels in the groups 4, 5 and 6 as compared with the control group. The increase was more in group 6 which were treated by 120 mg of SB as compared with the control and the other groups. SB causes alteration in the functions of liver and kidneys which were appeared by occurrence of significant elevations in serum AST, ALT, urea, uric acid and creatinine. These results were agreed with that of (20). The SB is metabolized in the liver, conjugated with glycine resulting into formation of hippuric acid which led to the depletion of glycine, that undergo many metabolites process causing decrease the levels of liver enzymes, urea, uric acid, creatinine and glutamine (21, 22). The elevation of serum ALT and AST reflect a liver damage because these enzymes assessed for liver function. In the human being, when given an oral dose of 60 mg \Kg BW, about 70% of the dose is excreted as hippuric acid after 3 hrs. and the rest excreted within 3 day (20). The AST and ALT are regarded as a key enzyme in the amino acid metabolism, a major liver function that is commonly applied as markers in assessing liver. They are reported that they have different activates in the level of the human erythrocyte. The ALP is present in several body tissue (Extrahepatic) and its level not specific for liver only. The coadministration of Vit.C causes significant reduction of the different parameters as compared with the control group. These results, in the present study regarding the levels of AST, ALT and ALP, that were seen in groups given Vit.C with SB, are in coincidence with that found by (23) and (24). Vit.C is water soluble (hydrophylic), considered as one of the important antioxidants trapping the free radicals in the extracellular fluid protecting the biomembrance from peroxidation damas. The effect of benzene resulted from the combination of (SB+Vit.C) on serum level of liver enzymes (ALT and AST) is significant ($P<0.001$) and $P<0.05$, respectively the increased of benzene concentration of benzene affected the ALT, AST and ALP level causing its reduction (25). The results of the present study as demonstrated in table (2) showed a significant increase of blood glucose in G6 as compared with the other groups and the control. (26) reported frequent consumption of soft drinks and other foods containing benzoate could increase the risk of

developing type 2 *Diabetic mellitus* other several reports in animals and from cell culture field suggested that SB and hippurate caused significant impact on glucose hemostasis (for example :I\V infusion of benzoic acid in sheep lead to elevation of serum glucose concentration, insulin and glucagon without concurrent glucose administration (27).

The SB and its metabolite Hippurate caused insulin and glucagon secretion as well as increasing the peripheral insulin action and causing significant impact on response to glucose challenge and represent as a potential through diabetes chronic environment exposure, these resulted reported by (28). It is also showed a significant decrease of total protein in G5 and G6 as compared with other groups and control. Some others reported that benzene causes energy and protein metabolism dysfunctions that may attach to microsomal proteins and hepatic enzymes (29). Significant increase in the serum albumin and total protein levels in rats treated with SB for 10 days (30). While (31) reported no significant changes in total protein while significant increase serum urea. The benzene formation from reaction of combination SB with Vit.C which inhibits the protein synthesis by inactivating some proteins and damaging DNA and RNA molecules (32). The results showed significant increase of blood urea in the groups 3 ,4,5 and 4 but the most significant increase was in G5 its also appeared that there is significant increase of creatinine in G5 and 6, but the most significant increase was in G6, regarding the blood urea and serum creatinine our study recorded that there is significant increase of their parameters in the group 4,5 and 6 as compared with the control and the other groups. These increments may be due to the histopathological changes that occurs in the kidneys, resulted into impairment of excretion of urea and increasing of its concentration in blood elevation of serum creatinine and adaption of total protein, which all indicated that there is defect in renal (kidney) function (33). Similar result was observed by (34) who reported that changes in albumin and total protein levels and signed to hepatocytes enlargement and glassy cytoplasm in peripheral urea of level in mouse and rats that fed SB in varying doses. (35) showed that using benzoic acid as preservation agent for juices. They added benzoic acid to drinking water for rats led to significant increasing total protein of the biochemical parameters such as globulin and albumin and also highly significant difference in values of uric acid, urea and

creatinine concentration. However, we find that sodium benzoate exposure significantly influences for circulating metabolism is in the range used in cell culture studies demonstrating an inhibitory effect of Hippocrates on glucose uptake (36). The significant elevation of serum urea, creatinine and uric acid by SB are indicative of damage of the kidneys, Urea, Uric acid and creatinine are waste product of metabolism. There are found in the liver and conveyed through blood to the kidney for excretion. The healthy kidney removes that compound from the blood to be excreted in the urine (37). In fact, there was no significant change in the total serum protein in the rat indicating that the toxicity of the drug was not significant enough to inhibit protein synthesis in the liver, and the lack of change observed in the concentration of this protein is an indication that the good bowel function in the rat was not affected. If gastrointestinal absorption is affected, there isn't enough amino acids absorbed and protein synthesis is impaired. The administration of Vit.C to adult male rats causes reduction of glucose level toward the normal values as compared with the control (38). The benzene effect on glucose and glycogen levels may be due to genetic damage caused by benzene. It was shown that benzene and its metabolites cause genetic damage (oxidative DNA) damage, numerical and structural chromosomal damage and micronuclei by (39).

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بنزوات الصوديوم الحافظة كمضافات غذائية لوحدها وممزوجة مع فيتامين ج وتأثيراتها السامة على وظائف الكبد والكلى لدى ذكور الأرانب البالغين

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الخلاصة

صممت الدراسة الحالية لتحديد التأثير الضار لبنزوات الصوديوم (SB) وفيتامين ج بمفردهما وممزوجين مع بعض على وظائف الكبد والكلى في الأرانب. تم تقسيم ستة وثلاثين ذكورًا بالغًا بشكل عشوائي إلى ست مجموعات متساوية. المجموعة 1 (مجموعة السيطرة) التي أعطيت ماء مقطرعن طريق الفم، المجموعة 2: فيتامين ج (200 ملغم / كجم من وزن الجسم / يوم) اعطيت عن طريق الفم، المجموعة 3: أعطيت عن طريق الفم بنزوات الصوديوم (60 ملغم / كجم من وزن الجسم / يوم) ،

المجموعة 4: اعطيت عن طريق الفم بنزوات الصوديوم (120 ملغم / كجم من وزن الجسم / يوم). المجموعة 5: اعطيت عن طريق الفم بنزوات الصوديوم وفيتامين ج (60 +200) ملغم / كجم من وزن الجسم / يوم) والمجموعة 6: اعطيت عن طريق الفم بنزوات الصوديوم وفيتامين ج (120+ 200 ملغم / كجم من وزن الجسم / يوم). أظهرت النتائج زيادة معنوية في انزيمات الكبد ALT وAST وALP وMDA في المجموعات 4 و5 و 6 بينما زادت مستويات البروتين الكلي في G3 و G4 وانخفضت في G5 و G6 مقارنة بمجموعة السيطرة والمجموعات المعالجة الأخرى. زادت مستويات اليوريا والكرياتينين في G5 وG6 مقارنة بمجموعات أخرى. نستنتج بإن تناول بنزوات الصوديوم بمفرده وممزوج مع فيتامين ج قد تسبب في آثار سلبية على وظائف الكبد والكلى مقارنة بالمجموعة التي عولجت بفيتامين ج وحده ومجموعة السيطرة.