

The Effect of Treating with Ascorbic Acid (C Vitamin) on some Biochemical Variables in the Blood of Male Rats Treated with Sodium Benzoate

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Abstract

The current study comprised on learning the prophylactic effect for C Vitamin or Ascorbic Acid (as its non-enzymatic food antioxidant) in some biochemical variables in white male rats (*Norvegicus Rattus*) age (3-4) months and used 18 once in this study. It was divided randomly into three groups each one comprised 6 rats. Consequently the first group was fed the distilled water by tube dosage and considered the control group, the second one fed Sodium Benzoate by dosage 50 mg /kg of body weight, and the third one fed Sodium Benzoate by dosage 50 mg/ kg b. w. with C vitamin dosage 100 mg/ kg b. w., and the swallowing has achieved daily for 30 days. The results have showed that the treating with Sodium Benzoate caused a moral decreasing in protein, albumin, and glutathione concentrations in the blood of male rats treated with the material in contrast with the control group as the decreasing was abdicable in time. Moreover, the treated results showed a moral increasing in concentration of uric acid, malondialdehyde, and transaminase enzyme activity (Amin Alanine transaminase (ALT) and Amin Aspartate transaminase (AST)) in the blood serum of the treated male rats in contrast with the control group. In addition, the results showed that the rats treated with C vitamin dosage 100 mg/ kg b. w. have a moral increasing in the level GSH and a moral decreasing in the level MAD in the blood serum of the treated animals in contrast with the control group beside its positive effect in some biochemical variables. Thus indicates that C vitamin has a prophylactic ability in removing the effects of oxidant stress and the adverse effects of sodium benzoate.

THE PREFACE

The material of sodium benzoate prepares from the reaction of benzoic acid with sodium hydroxide, and it is a sold material and has a molecular weight is (144.12), melting point is 430-440 °C, density is 1.44 g/mole ^{(1) (2)}. The material of sodium benzoate finds in mulberry naturally and it used as a preservative material in soft and non-soft drinks, toothpaste, spices, pickles, juices, and some food ⁽³⁾. Also, the sodium benzoate used in cosmetic of make up as 0.02-0.05 % and in injected fluids as 0.1-0.5 %. Otherwise, what determine it as a preservative material that is the acidic medium at PH hydrogen index lower than 4. However, it is preferably used sodium or potassium benzoate instead of benzoic acid because the soluble in water is more than the benzoic acid ⁽⁴⁾.

Sodium benzoate is used to investigate liver functions in the case of neonatal children ⁽⁵⁾ that it working on combine with glycine inside the body to give the hippic acid so the glycine concentration in body decreases because the combination of sodium benzoate and the acid discharges out of the body through kidney with urea ⁽⁶⁾.

Sodium benzoate is used to reduce the blood plasma and treating the patients whom suffering of metabolic disorder in urea circle ⁽⁷⁾. The maximum of eating sodium benzoate should be 5 mg/ kg from the body weight as determined by Food & Drug Administration (FDA).

The mechanical of using Sodium Benzoate in acidic medium belongs to formation of benzoic acid that ascribe to it as stopping the growing of bacteria as it absorbs inside the bio cell which works on decreasing the glucose anaerobic fermentation through phosphofructokinase enzyme in rate 95 % which works on change the material of 6- phosphate to fructose 1,6 bi-phosphate ⁽⁸⁾.

The research aims to study the effect of ascorbic acid or C vitamin with sodium benzoate that responsible on formation the free roots and occurrence the oxidant stress

which is considered an activated factor for oxidant as it is pro-oxidant lead to increasing of fat peroxide and damaging the cellular membrane cause of losing the membranes flexibility, increasing their permeability, and changing their functions completely. So, the study aims to discover the adverse effects of sodium benzoate and the positive effects of C vitamin with sodium benzoate in some variables in the blood of laboratory's Rats.

THE MATERIALS AND METHODS

The Animals

Preparation of Animals:

It be used in the current study 24 white male rats from the kinds of *Rattus Norvegicus* at the age of (3-4) months with weight of (200-250) gm., and all of them were good healthy obtained from Mosul Medical College in Mosul University. The rats were growing in plastic boxes under the standard circumstances as 14 hours light and 10 dark in a day, the natural light, the temperature was (3±25°) with ventilation, and the animals were given the feed and natural water in free form, then the animal divided into groups randomly.

The Experimental Designing:

Sodium benzoate was used as a white powder to prepare the test fluid in order to obtain the swallowed liquid, also it was used C vitamin (ascorbic acid) obtained it from General Company for Drugs and Medical Appliances in Nineveh to use it as an out source alimentary anti-oxidant. Then, the animals divided randomly into three groups as 6 rats for each one, put them in separated boxes, and treated as in the following:

- Control group: this group included non-treated animals which feed the standard provender with drinking water along the swallowing period, and it has fed distilled water by using the gavage tube to balance the animal stress and holding.

- Group of sodium benzoate: this group was swallowed dose 50 mg/kg from body weight by using the tube for 30 days ⁽⁹⁾.
- Group of sodium benzoate with C vitamin: this group was swallowed by dose of sodium benzoate as 50 mg/kg from body weight with C vitamin as 100 mg/ kg from body weight daily for 30 days.

Samples' Collection:

After 15 and 30 days from treating each once lonely, it has got the blood from eye socket of each rat by capillary tubes, the blood has put in dry plastic tubes which have compacted covers and empty from any anti-coagulant, left them under the degree of room temperature for 20 minutes until the blood coagulated, then centrifuged them at speed 300 circle/ minute for 10 minutes to get the blood serum for the chemo-biological investigations.

The serum used to exam the levels of some different chemo-biological variables by using different test methods.

Test of some Chemo-biological Variables:

Also, it has used some of prepared investigations from French Companies Biolabo and Biomerieux to test the concentrations of the following variables: total protein, albumin, uric acid, and activity of ALT and AST enzymes, whereas the other rest variables have used the manual methods to test them.

The Statistical Analysis:

The results have analyzed among the control group and the other study groups by using TUnpaired test and the results considered moral at the probability level ($P \leq 0.05$) and expressed them by the rate \pm the standard false.

RESULTS AND DISCUSSION:

The results in the table (2) show that the treating of the rats with sodium benzoate in dose 50 mg/ kg from the body weight led to moral decreasing in the level of glutathione in the serum blood of male rats in different periods in contrast with the control group, and this study harmonizes with the results of former study concluded that giving sodium benzoate to the patients of renal failure led to decreasing the glutathione after 90 and 180 minutes from the swallowing operation ⁽¹⁶⁾. The declining became 9 % after 30 days from the treating, the decreasing may ascribe to the glutathione effective participation as anti-oxidant non-

enzymatic (internal formation) in prevention the oxidation updating with sodium benzoate either through the direct removing to the free roots, or by the enzymes that the glutathione (GSH) represents the defensive border against the toxicity of any material inter in number of cellular mechanisms represented by the cellular defense against the toxicity of number of compounds and drugs with oxidant stress.

The food preservative materials such as sodium benzoate have a frustration role to the several of enzymes important for reforming GSH the reduced form of the oxidant once such as GSSG and glucose phosphate dehydrogenase activity towards phosphate group causing frustration to the pentose phosphate pathway causing the reduced form to the NADPH enzyme ⁽¹⁷⁾.

Moreover, the results in table (2) showed that the treating the rats with sodium benzoate in dose 50 mg/ kg from the body weight led to moral increasing in malondialdehyde (MAD) in rate 32 % and 44 % from the rats' blood serum treated at the periods for 15 and 30 days successively. Thus, refers to increase the roots' producing oxidant to the oxygen stem which effect on the cellular membranes during the increasing of fat peroxide, damaging the cellular membranes, increasing the amount of phosphatic fat, in addition to oxygen deficiency, thus what Yong et al., stated ⁽¹⁸⁾ through giving the sodium benzoate to the rabbits suffering of renal failure led to decline the level of malondialdehyde for the treated animals in contrast with the untreated once caught by renal failure.

However, the results in table (2) are showed that the treating animals by C vitamin in dose 100 mg/ kg from the body weight led to moral decreasing in the level of malondialdehyde in corresponded with moral increasing in the level of glutathione, beside the results of table (2) showed that the decreasing and increasing were more obvious after the period of 30 days to the swallowing, since C vitamin considers as non-enzymatic self-discipline in the water (exterior source) anti-oxidant. It operates on protection the plasmatic membranes to the cells and exterior shell to the lipped proteins from damaging by lipid peroxide in phosphatized lipids of the plasmatic membrane; also it works on removing the affective kinds of oxygen ⁽¹⁹⁾ ⁽²⁰⁾.

Variable Tested	Method	The Source
Total Protein concentration	Biuret method	⁽¹⁰⁾
Albumin concentration	Bromocresol green method	⁽¹¹⁾
Uric acid	Enzymatic method	⁽¹²⁾
Amin Alanine Transaminase activity (ALT)	Colourimetric method	⁽¹³⁾
Amin Aspartate Transaminase activity (AST)	Colourimetric method	⁽¹³⁾
Malondialdehyde level (MDA)	Thiobarbituric acid (TBA)	⁽¹⁴⁾
Glutathione level (GSH)	Modified Ellman's reagent method	⁽¹⁵⁾

Table (1): The test methods used to investigate some of chemo-biological variables in current study.

Table (2) shows the effect of C vitamin in the concentration of Malondialdehyde & Glutathione in the blood serum of the male rats treated with Sodium Benzoate in different periods.

The Tested Variables	Glutathione concentration (micromole/liter)						Malondialdehyde concentration					
	15 days			30 days			15 days			30 days		
The Groups	The rate ± standard false	% for concentration	% for change	The rate ± standard false	% for concentration	% for change	The rate ± standard false	% for concentration	% for change	The rate ± standard false	% for concentration	% for change
Control	0.16±18.8	100	-	0.16±18.07			0.16±2.19	100		1.8±2.19	100	-
Sodium Benzoate in dose 50 mg/kg from body weight	0.23±15.5 ⁵ ****	82	- 12	0.21±14.6 ⁶ ****	78	- 22	0.13±2.9*	132	32	0.13±4.2 ^{***}	144	44
Benzoate dose 50 mg/kg & C Vitamin in dose 100 mg/kg from body weight	0.27±18.4	102	+ 2	0.10±17	109	+9	0.10±1.09	86	-14	0.2±1.7*	77	-33

The rate & standard false for six repeated.

The number of animals for each group = 6

* Refers to moral difference at the level (0.05 ≥ p).

** Refers to moral difference at the probable level (0.01 ≥ p).

*** Refers to moral difference at the probable level (0.001 ≥ p).

Table (3) shows the effect of C vitamin in the concentration of Protein, Albumin, and Uric acid in the blood serum of the male rats treated with Sodium Benzoate in different periods.

The Tested Variables	Protein (mg/ 100 ml)		Albumin (mg/ 100 ml)		Uric acid (mg/ 100 ml)	
	15 days	30 days	15 days	30 days	15 days	30 days
The Groups	The rate \pm standard false	The rate \pm standard false	The rate \pm standard false	The rate \pm standard false	The rate \pm standard false	The rate \pm standard false
Control	0.14 \pm 6.4	0.14 \pm 6.4	0.2 \pm 4.2	0.2 \pm 4.2	0.18 \pm 3.8	0.18 \pm 3.8
Sodium Benzoate in dose 50 mg/kg from body weight	0.18 \pm 5.09**	0.13 \pm 5.7	0.22 \pm 3.8**	0.17 \pm 3.3***	0.21 \pm 4.1	0.24 \pm 4.8***
Sodium Benzoate dose 50 mg/ kg & C Vitamin in dose 100 mg/ kg from body weight	0.19 \pm 6.1**	0.24 \pm 6.18	0.14 \pm 3.5*	0.27 \pm 4.01	0.13 \pm 3.89**	0.12 \pm 3.7

The rate & standard false for six repeated.

The number of animals for each group = 6

* Refers to moral difference at the level (0.05 \geq p).

** Refers to moral difference at the probable level (0.01 \geq p).

*** Refers to moral difference at the probable level (0.001 \geq p).

Table (4) shows the effect of C vitamin in the concentration of ALT & AST in the blood serum of the male rats treated with Sodium Benzoate in different periods.

The Tested Variables	Combating ALT enzyme in blood serum (IU/L)						Combating AST enzyme in blood serum (IU/L)					
	15 days			30 days			15 days			30 days		
Treating Period	The rate \pm standard false	% for concentration	% for change	The rate \pm standard false	% for concentration	% for change	The rate \pm standard false	% for concentration	% for change	The rate \pm standard false	% for concentration	% for change
Control	2.12 \pm 48.4	100	-	48.4 \pm 2.12	100	-	3.25 \pm 85.98	200	-	3.23 \pm 85.98	100	-
Sodium Benzoate in dose 50 mg/kg from body weight	0.25 \pm 61.16*	126	26	0.22 \pm 66.19***	136	36	4.5 \pm 129.38*	150	50	4.65 \pm 135.3	157	57
Sodium Benzoate dose 50 mg/ kg & C Vitamin in dose 100 mg/ kg from body weight	3.23 \pm 49.98	103	3	0.14 \pm 46	95	-5	2.12 \pm 84.49*	98	-2	0.5 \pm 82.412	9500	-5

The rate & standard false for six repeated.

The number of animals for each group = 6

* Refers to moral difference at the level (0.05 \geq p).

** Refers to moral difference at the probable level (0.01 \geq p).

*** Refers to moral difference at the probable level (0.001 \geq p).

The results in table (3) showed that the influence of Sodium Benzoate in total protein and albumin was mild so slight declining in protein level happened without any change in albumin level refers that the Sodium Benzoate works on frustration protein creating in the liver⁽²¹⁾.

Whereas the treating of the rats with C vitamin led to high total protein and albumin concentration in contrast with the control group, and the reason for this action is the C vitamin works on stimulating the operation of creating both the protein and albumin in the liver that agree with Hamed⁽²²⁾ when giving C vitamin to the rats which treated with aluminum sulphate and the role of C vitamin through preventing fat peroxide in cellular membranes and protecting them damage to give the organs (liver & kidney) chance to achieve their biological function truly such as proteins creating⁽²⁰⁾.

Moreover, the results in table (3) are showed to the high level of uric acid when treating the rats with sodium benzoate by dose 50 mg/ kg from body weight whereof indicates that there is damage in kidney's tissues and fail them for the health kidney discharges the uric acid in urine as it one of the toxic materials⁽²³⁾, since the uric acid considers the last conclusion for demolition the boranes and damaging the peptide bonds, in addition uric acid considers non-enzymatic antioxidant (interior source) that ascribe to fail of the kidney's tissues from discharging it because the destroying of these tissues⁽²⁰⁾.

Besides, the results of table (3) are showed C vitamin by dose 100 mg/ kg from the body weight led to decreasing uric acid in blood serum to the level approximate from the control group and these results agree with the results of⁽²⁴⁾ that is C vitamin works on decreasing the concentration of uric acid for the rats treated with sodium sulphate.

The damage in liver has diagnosed from the transporter enzymes activity test (ALT & AST), so the study focused on the activity of these two enzymes for they consider as an indicator of damage occurs due to sodium benzoate in liver tissue. The results in table (4) demonstrated a moral increasing in activity of enzyme Amin alanine transaminase (ALT) and enzyme Amin Aspartate transaminase (AST) in the serum of rats treated with sodium benzoate in dose 50 mg/ kg from the body weight in contrast with the control group, then the highest rate in the activity of enzyme ALT was 36 % after 30 days, whereas the rate of AST was 35 % after 30 days and this what is referred by Wang and Srira⁽²⁵⁾ that sodium benzoate works on increasing the levels of the two enzymes ALT and AST in the blood serum exposing to it. Then it leads to accrete this material in liver tissues causing poisoning them and destroying of the liver cells, then leads to release these enzymes through the decaying liver cells to the circulation of the blood. This indicates to the range of the damage occurs in liver tissue.

Whereas the results in table (4) refers that the treating with C vitamin by dosage 100 mg / kg from the body weight led to decreasing of the enzymes ALT and AST activities in contrast with the control group. The role of C vitamin ascribes to prevent the attack of the free roots to cellular membranes and prevent the defect penetration these membranes in liver⁽²⁸⁾.

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