RESEARCH ARTICLE

Antioxidant Role of Methionine: As an Essential Sulfur-containing Amino Acids

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ABSTRACT

Methionine is one of the essential sulfur-containing amino acids that are used in building proteins. In the body, methionine condenses with ATP to form S-adenosylmethionine (SAM), which acts as a methylation donor in various biological pathways. Methionine is characterized by its antioxidant activity and its ability to modify tissue sensitivity against oxidizing agents. Therefore, this study's aim included using hydrogen peroxide 0.5% in drinking water to induce oxidative stress in the male rats and testing the ability of different concentrations of methionine for protection or prevention the oxidative stress during 10, 20 and 30 days.

Forty male rats with the age of 3-4 months and of weights ranging between 300 to 400 gm were divided into 4 groups: Group (1): control group received drinking tap water, group (2): treated with H_2O_2 0.5% in drinking water, group (3): treated with H_2O_2 and methionine 0.3%, group (4): treated with H_2O_2 and methionine 0.6%. The following parameters in the serum were measured: Vit. C, Vit. E, peroxynitrite, albumin, selenium, zinc, and copper.

Treatment with 0.3% methionine produced clear effects on the vit C, peroxynitrite, Zn and Cu levels in the serum, while the treatment with 0.6% methionine produced clear effects on the serum vit E, albumin, and Se levels.

Keywords: Methionine, S-adenosylmethionine, Antioxidant sulfur-containing, Compounds

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INTRODUCTION

Methionine is one of the essential sulfur-containing amino acids that are used in building proteins. Methionine is a source of sulfur atom for many compounds in the body that are needed for metabolism and normal growth, such as cysteine, taurine, carnitine, and glutathione through the sulfur transport pathway. In the body, methionine condenses with ATP to form the compound S-adenosylmethionine (SAM), which is called active methionine. SAM acts as a methylation donor in various biological pathways known as methylation reactions, which are essential in cartilage synthesis and the removal of toxic metabolites.[1] SAM helps to improve normal liver function, as it is used in European countries to treat cases of cirrhosis[2] and liver damage caused by alcohol and in the treatment of acetaminophen poisoning[3] SAM is used to protect the stomach from bleeding resulting from the use of non-steroidal drugs used in the treatment of arthritis, including aspirin and naproxen. Methionine have been shown to reduce the dose and side effects of some anticancer drugs.[4] Methionine is essential in determining the levels of sulfur-containing compounds, including glutathione.[5] Studies indicate that SAM is beneficial for many mental illnesses, including depression, Parkinson's disease, and Alzheimer's.[6] It was noted that the level of SAM decreased in these patients, and the level of methionine was reduced in AIDS patients. The researcher[7] indicated that the use of 5 gm supplement of methionine daily, helps treat some of the symptoms of Parkinson. The researcher[8] also indicated that supplying 6g of methionine daily helps in improving memory recall for AIDS patients with nervous system disorders. Methionine is characterized by its antioxidant activity and its ability to modify tissue sensitivity to oxidizing agents. Therefore, the study aimed to use methionine to reduce oxidative stress and enhance the endogenous antioxidants in rats exposed to oxidative stress induced by hydrogen peroxide.

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MATERIALS AND METHODS

Hydrogen Peroxide Preparation

Hydrogen peroxide (H_2O_2) was used at a concentration of 50% supplied by (Laboratory Reagents, India) and diluted with normal drinking water to a concentration of 0.5%.[9] The solution was prepared daily and given to the experimental animals in special bottles prepared for drinking water for the duration of the experiment of 30 days.

Preparation of Methionine

The amino acid DL-methionine, supplied by (BDH Laboratory Reagent Chemicals Ltd, Poole, England) was used. Concentrations of 0.3 and 0.6% were prepared daily and given to the experimental animals in the bottles of drinking water for 30-day experimental period.

Experimental Animals

In 40 adult albino male rats aged 3 to 4 months, with weights ranging 300 to 400 gm, were used. The animals were housed in the animal house of Veterinary Medicine College, University of Mosul in a special room with the optimum conditions for feeding, temperature, lighting, and ventilation. The animals were divided into 4 groups of 10 animals for each group and treated for 30 days (the duration of the experiment) as follows:
Group (1): given normal drinking water and was considered a control group.
Group (2): given drinking water containing 0.5% hydrogen peroxide.
Group (3) : given drinking water containing 0.5% hydrogen peroxide and 0.3% methionine.
Group (4): given drinking water containing 0.5% hydrogen peroxide and 0.6% methionine.

Blood Collection

Blood samples were collected from animals during the periods before treatment (time zero) and after 10, 20 and 30 days of treatment. Blood was withdrawn from the eye vein by means of a capillary tube containing heparin implanted in the inner corner of the eye socket. Two serum samples were combined into one group, bringing the total number of each group to 5. Serum samples were kept in frozen for the subsequent studies.

Chemical tests

Vitamin C was estimated using the method explained by.[10] The method explained by[11] was used to estimate vitamin E. Peroxynitrite radical based on the researchers' modified method.[12] The amount of albumin was estimated using the green bromocresol method, which used ready-made solutions (kit) supplied from the Syrian Syrbio company. Method used by an author[13] was used to estimate the selenium concentration. The serum's zinc and copper level was estimated using atomic absorption spectroscopy.[14]

Statistical Analysis

The data were analyzed using the two-way analysis of variance. Duncan test was used to determine the statistical differences between the different groups, and the significant difference for all the tests was at the level of probability ($p < 0.05$).[15]

RESULTS AND DISCUSSION

Effect of Methionine on Vitamin C Level

The results in Table 1 showed that 0.5% of hydrogen peroxide in drinking water decreased the vitamin C level in all treatment periods compared to the control group. The treatment with methionine led to an increase the vitamin level in all the treatment periods compared with the group treated with hydrogen peroxide, in addition, the concentration of 0.3% of methionine showed a significant increase in the vitamin C level compared with the control value.

These results are consistent with that found by a researcher,[16] who mentioned that methionine works to raise the level of vitamin C to a normal level when administered orally to rats suffering from oxidative stress as a result of a deficiency in vitamin B6. The mechanism of antioxidant action of methionine was explained as that many oxidants interact directly with methionine to form the methionine sulfoxide. Thus, methionine residues in the proteins provide a high concentration of reactants that act as traps for active oxygen species.[17]

Effect of Methionine on Vitamin E Level

The results in Table 2 indicated a significant decrease in vitamin E level in the group treated with hydrogen peroxide during the 20 and 30 days of treatment compared with the zero period and the control group. This result clarifies the role of oxidants, including hydrogen peroxide, in causing oxidative damage through the release of free radicals, which often accompany the oxidation of fats and damage to channels and ion pumps in the membranes, which result to the destruction of cellular membranes and lipoproteins in which vitamin E is concentrated, leading to its destruction and consequently decrease its level in the blood and tissues.

Concentration of 0.6% of methionine led to a significant increase in vitamin E level in 10 and 30 days of treatment compared with its counterparts of hydrogen peroxide treated group. This result corresponds to what the researcher[18] concluded: giving 10 mg/kg of methionine or SAM daily for 9 weeks to the male rats suffering from cirrhosis induced by CCl4 led to a significant increase in vitamin E level in the blood. The researcher[18] explained the role of methionine in restoring the glutathione, the reduced glutathione reactivates the oxidized form of vitamin E, which results from the process of lipid peroxidation in cell membranes, thus protecting it from the breakdown caused by lipid peroxidation.[19-24]

Effect of Methionine on Peroxynitrite Radical

The results in Table 3 showed that treatment with H_2O_2 led to increase the peroxynitrite radical in the treatment periods of 10 and 30 days compared with its counterparts in the control group, these results are consistent with results[25] where it indicated a significant increase in the peroxynitrite radical in the liver of cows treated with hydrogen peroxide.

The different concentrations of methionine showed a significant decrease in the level of peroxynitrite in all treatment periods compared with its counterparts in the hydrogen

Table 1: Effect of methionine in the level of serum vitamin C

Treatments	*Vitamin C concentration (µmol/L)*			
	Treatment duration/day			
	0	*10*	*20*	*30*
Control	64.96 ± 1.32$^{d\ e}$	60.67 ± 1.31$^{e\text{-}j}$	57.93 ± 1.65$^{i\ j\ k}$	63.19 ± 1.04$^{d\text{-}g}$
H_2O_2 0.5%	61.29 ± 1.49$^{e\text{-}j}$	52.74 ± 1.93$^{l\ m}$	42.36 ± 1.66^{n}	39.68 ± 1.93^{N}
H_2O_2 0.5% + methionine 0.3%	55.74 ± 1.29$^{k\ l}$	72.49 ± 1.66$^{a\ b}$	73.99 ± 1.12^{a}	61. 95 ± 1.54$^{e\text{-}i}$
H_2O_2 0.5% + methionine 0.6%	60.43 ± 1.32$^{f\text{-}j}$	58.60 ± 1.93$^{h\text{-}k}$	64.28 ± 1.80$^{d\ e\ f}$	52.75 ± 1.59$^{l\ m}$

Values was expressed as the mean of five animals ± standard error. The different letters in each row and column indicate the presence of significant differences at ($p < 0.05$).

Table 2: Effect of methionine on vitamin E level

Treatments	*Vitamin E concentration (µmol/L)*			
	Treatment duration/day			
	0	*10*	*20*	*30*
Control	20.73 ± 0.47^{a}	19.66 ± 0.74$^{a\ b\ c}$	19.50 ± 1.18$^{a\ b\ c}$	19.50 ± 1.18$^{a\ b\ c}$
H_2O_2 0.5%	19.94 ± 0.66$^{a\ b\ c}$	17.72 ± 0.93$^{c\ d}$	16.87 ± 0.33^{d}	16.74 ± 0.59^{d}
H_2O_2 0.5% + methionine 0.3%	20.60 ± 0.22^{A}	19.94 ± 0.28$^{a\ b\ c}$	18.18 ± 0.17$^{b\ c\ d}$	18.06 ± 0.12$^{b\ c\ d}$
H_2O_2 0.5% + methionine 0.6%	19.41 ± 0.92$^{a\ b\ c}$	20.23 ± 0.58$^{a\ b}$	18.63 ± 0.28$^{a\text{-}d}$	19.43 ± 0.41$^{a\ b\ c}$

Values are expressed as the mean of five animals ± standard error. The different letters in each row and column indicate the presence of significant differences ($p < 0.05$).

peroxide-treated group. However, the concentration of 0.6% showed a significant increase in peroxynitrite in the 30-day treatment period compared with the control values. The researcher[26] has indicated a decrease in Peroxynitrite radical level in methionine-treated bovine serum.[27] referred to the antioxidant role of the intermediate compounds produced during the metabolic pathway of converting methionine to taurine. It was shown that cysteine sulphonic acid has the same protective properties of hypotaurine against the damage caused by ONOO radical, and this indicates the role of sulfinic group, which is oxidized to a sulfonate group during the inhibition of peroxynitrite radical and its derivatives.

Effect of Methionine on Serum Albumin Level

Treatment with H_2O_2 led to a significant decrease in the level of albumin in the periods 20 and 30 days of treatment compared to its counterparts in the control group and zero period for the same group (Table 4).

Table 3: Effect of methionine on peroxynitrite radical

Treatments	*Peroxy nitrite concentration (µmol/L)*			
	Treatment duration /day			
	0	*10*	*20*	*30*
Control	49.26 ± 2.21$^{f\text{-}j}$	44.77 ± 1.33$^{j\ k\ l}$	52.34 ± 1.89$^{b\text{-}f}$	45.73 ± 1.27$^{h\ l}$
H_2O_2 0.5%	48.86 ± 1.44$^{f\text{-}j}$	62.49 ± 2.26^{a}	56.10 ± 1.50^{b}	54.58 ± 1.39$^{b\ c\ d}$
H_2O_2 0.5% + methionine 0.3%	50.27 ± 1.26$^{d\text{-}h}$	43.74 ± 1.63$^{k\ l\ m}$	43.03± 1.89$^{k\ l\ m}$	42.90 ± 1.76$^{k\ l\ m}$
H_2O_2 0.5% + methionine 0.6%	44.94 ± 1.49$^{i\text{-}l}$	49.40 ± 1.61$^{f\text{-}i}$	42.26 ± 1.88$^{k\ l\ m}$	56.35 ± 1.42^{B}

Values are expressed as the mean of five animals ± standard error. The different letters in each row and column indicate the presence of significant differences ($p < 0.05$)

Table 4: Effect of methionine on serum albumin level

Treatment	*Albumin concentration (g/dL)*			
	Treatment duration /day			
	0	*10*	*20*	*30*
Control	4.39 ± 0.14$^{b\ c\ d}$	4.03 ± 0.24$^{d\text{-}g}$	4.34 ± 0.076$^{b\ c\ d}$	4.35 ± 0.15$^{b\ c\ d}$
H_2O_2 0.5%	4.40 ± 0.15$^{b\ c\ d}$	4.27 ± 0.093$^{c\ d\ e}$	3.57 ± 0.10$^{i\ h}$	3.37± 0.090^{i}
H_2O_2 0.5% + methionine 0.3%	4.10 ± 0.11$^{c\text{-}g}$	4.69 ± 0.17$^{a\ b}$	4.22 ± 0.60$^{c\ d\ e}$	3.72 ± 0.50$^{g\ h}$
H_2O_2 0.5% + methionine 0.6%	4.14 ± 0.20$^{c\ d\ e}$	4.96 ± 0.50^{a}	4.68 ± 0.60$^{a\ b}$	3.96 ± 0.11$^{e\ f\ g}$

Values are expressed as the mean of five animals ± standard error. The different letters in each row and column indicate the presence of significant differences ($p < 0.05$)

The different concentrations of methionine showed a significant increase in the level of albumin in all treatment periods compared with its counterparts in the H_2O_2 -treated group. However, the 0.6% concentration showed a clear effect in the 10 and 20 days of treatment compared with the zero period for the same group. In a study about the role of cysteine and methionine in human serum albumin, it was shown that cysteine and methionine residues constitute 40 to 80% of the albumin components, and the methionine in albumin acts as a metal chelating agent , while the cysteine acts as a scavenger of free radicals.[28]

Effect of Methionine on the Selenium Level

Treatment with H_2O_2 showed a significant decrease in selenium concentration in all treatment periods compared with the control group and zero period for the same group, and this is consistent with the results of[29] that oxidative stress conditions prevent the absorption of selenium by the gastrointestinal tract due to the damage occurs in the endothelial cells of the intestine (Table 5).

Table 5: Effect of methionine on the selenium level

Treatment	*Selenium(Se) concentration (µmol/L)*			
	Treatment duration /day			
	0	*10*	*20*	*30*
Control	0.28 ± 0.050$^{a\ b}$	0.31 ± 0.050^{a}	0.29 ± 0.074$^{a\ b}$	0.25 ± 0.020$^{c\text{-}f}$
H_2O_2 0.5%	0.24 ± 0.090$^{d\ e\ f}$	0.18 ± 0.010^{l}	0.13 ± 0.010^{J}	0.14 ± 0.060^{j}
H_2O_2 0.5% + methionine 0.3%	0.27 ± 0.010$^{b\text{-}e}$	0.24 ± 0.012$^{e\ f\ g}$	0.20 ± 0.074$^{g\ h\ i}$	0.28 ± 0.020$^{a\ b\ c}$
H_2O_2 0.5% + methionine 0.6%	0.24 ± 0.013$^{d\ e\ f}$	0.22 ± 0.080$^{f\ g\ h}$	0.20 ± 0.090$^{g\ h\ i}$	0.30 ± 0.020$^{a\ b}$

Values are expressed as the mean of five animals ± standard error.
The different letters in each row and column indicate the presence of significant differences ($p < 0.05$)

Table 6: Effect of methionine on the zinc level

Tratment	*Zinc(Zn) concentration (µmol/L)*			
	Treatment duration /day			
	0	*10*	*20*	*30*
Control	18.35 ± 0.55$^{c\ d\ e}$	16.21 ± 0.41$^{d\text{-}g}$	16.36 ± 0.49$^{d\text{-}g}$	15.75 ± 0.35$^{d\text{-}g}$
H_2O_2 0.5%	16.97 ± 0.52$^{d\ e\ f}$	18.35 ± 0.25$^{c\ d\ e}$	16.36 ± 1.54$^{d\text{-}g}$	10.85 ± 0.56^{i}
H_2O_2 0.5% + methionine 0.3%	19.72 ± 0.46$^{b\ c\ d}$	19.57 ± 0.48$^{b\ c\ d}$	24.16 ± 0.37^{a}	22.94 ± 0.20$^{a\ b}$
H_2O_2 0.5% + methionine 0.6%	16.21 ± 1.81$^{d\text{-}g}$	15.46 ± 0.40$^{e\ f\ g}$	13.0 ± 0.96$^{f\text{-}i}$	18.35 ± 0.55$^{c\ d\ e}$

Values are expressed as the mean of five animals ± standard error.
The different letters in each row and column indicate the presence of significant differences ($p < 0.05$)

Treatment with methionine showed a significant increase in the selenium concentration in all treatment periods compared with its counterparts in H_2O_2 treated group, and the concentration 0.6% showed a clear effect as it showed a significant increase in the 10 and 30 days of treatment compared with its counterparts in control group, These results are consistent with the results of[30] Which indicated the effect of dietary methionine on the selenium level and glutathione peroxidase (GSH-PX) activity in groups of rats fed diets equipped with different concentrations of selenium 0.007 to 0.5 mg/kg and 4 g/kg of methionine, he noticed a significant increase in the selenium level and GSH-PX activity in the blood and liver compared with the groups that were fed on diet equipped with selenium only.

Effect of Methionine on the Level of Zinc

The results in Table 6 indicated a significant decrease in the level of zinc in the 30 days of H_2O_2 treated group compared with its counterpart in the control group and the zero period for the same group.

Table 7: Effect of methionine on the copper level

Treatment	*Copper (CU) concentration (µmol/L)*			
	Treatment duration/day			
	0	*10*	*20*	*30*
Control	23.09 ± 0.42$^{a\text{-}d}$	23.72 ± 0.42$^{a\ b\ c}$	23.09 ± 0.40$^{a\text{-}d}$	24.50 ± 0.79$^{a\ b}$
H_2O_2 0.5%	22.46 ± 0.40$^{b\text{-}e}$	21.36 ± 1.57$^{d\text{-}g}$	18.53 ± 0.88^{h}	19.48 ± 0.71$^{g\ h}$
H_2O_2 0.5% + methionine 0.3%	21.36 ± 0.23$^{d\text{-}g}$	23.88 ± 0.27$^{a\ b\ c}$	24.50 ± 1.12$^{a\ b}$	24.50 ± 1.12$^{a\ b}$
H_2O_2 0.5% + methionine 0.6%	21.83 ± 0.26$^{c\text{-}f}$	23.72 ± 0.25$^{a\ b\ c}$	19.95 ± 0.36$^{f\ g\ h}$	21.83 ± 0.42$^{c\text{-}f}$

Values are expressed as the mean of five animals ± standard error.
The different letters in each row and column indicate the presence of significant differences ($p < 0.05$)

Methionine at a concentration 0.3% had a clear effect on the level of zinc, as it was able to raise the level of zinc in the periods of 20 and 30 days of treatment compared to its counterparts in the H_2O_2-treated group and the control group. The study of[31] indicated that the amino acids histidine and methionine work to increase the body's supply of dietary zinc, and their positive effect appears on the absorption of zinc from the intestine through its behavior as cheating agents.

Effect of Methionine on the Level of Copper

Treatment with H_2O_2 led to a significant decrease in the level of copper in all treatment periods, compared with its counterparts in the control group. The concentration 0.3% of methionine was able to raise the level of copper in all treatment periods compared with its counterparts in the H_2O_2-treated group for the same group (Table 7). A study explained by[32] about the role of methionine as a chelating agent of copper when given at a concentration of 0.05 M with drinking water for 42 days, he noticed that the level of copper decreased in the brain, liver, kidney, and heart tissues.

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