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# Protective effect of Ginger against Sodium Metabisulfite induced Oxidative Stress in Rat

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**Abstract**

Although sulfiting agents including sodium metabisulfite (SMB) are commonly used as preservative in foods, medicines and beverages, they have also been considered as important risk factors for the initiation and progression of diseases due to oxidative damage. The purpose of this report was to investigate the effect of ginger extract on serum oxidative stress indices and biochemical markers of liver and kidney function in SMB-treated rats. Twenty-four male Wistar rats (200-250 g) were divided into four groups: distilled water, ginger (500 mg/kg/day), sodium metabisulfite (260 mg/kg/day), and sodium metabisulfite + ginger. After 28 days of treatment, serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP) , total and direct bilirubin, creatinine, BUN, and total protein and antioxidant enzyme activities including glutathione peroxidase (GPx), glutathion reductase (GR), catalase (CAT) activities, and the levels of glutathione were tested. Differences in parameters among the four groups were assessed by one-way analysis of variance followed by Tukey's test. SMB ingestion resulted in a significant rise in serum liver enzymes (SGOT, SGPT, ALP). Serum antioxidant enzymes (GPx, GR, CAT), and the levels of glutathione were significantly decreased. However, ginger extract supplementation to the SMB treated rats partly reversed these effects to normal levels. Based on these results, sulfite induced oxidative stress was attenuated by ginger extract treatment, thus ginger can be used as a regular protective nutrient.

**Keywords**: Ginger, liver enzymes, Sodium metabisulfite, Oxidative stress.

## 1. Introduction

Sulfiting agents including sulfur dioxide and various sulfite salts are widely used as preservatives in pharmaceuticals and food industry. Despite their common uses, sulfites are toxic molecules and can react with a variety of cellular components including proteins, lipids, DNA, etc (Meng et al., 2004, Yi et al., 2005). Adverse effects of sulfite compounds in multiple organs of mammals including the pulmonary system (Vally and Misso, 2012) and the reproductive system (Rezaee et al. 2016, Shekarforoush et al., 2015) have been reported. An immediate increase in reactive oxygen species (ROS) production accompanied by a depletion of intracellular ATP followed by exposure of kidney cells and PC12 cells to sulfites was observed (Vincent et al., 2004, Zhang et al., 2004). Induction of lipid peroxidation was reported in the kidney and liver of rats treated orally with sodium metabisulfite (SMB) at a dose of 520 mg/kg/day (Elmas et al., 2005). Sulfites have been documented to alter the oxidant and antioxidant balance in rat erythrocyte (Ozturk et al., 2010) and serum (Shekarforoush et al., 2018).

Herbal medicines have received considerable attention over the last decades due to their diverse antioxidant activities. Ginger (Zingiber officinale Roscoe) is an herbal medicinal product with anti-tumorigenic, anti-inflammatory, and antioxidative activity (Ali et al., 2008). All ginger's major active ingredients, such as zingerone, gingerdiol, zingibrene, gingerols, and shogaols have anti-oxidant activities (Sakr and Badawy, 2011). In the alternative and folk medicine in the world, ginger has been used since antiquity to treat diseases like cold, headaches, nausea, gastrointestinal disturbances, rheumatic complaints, parasitic infections, and muscular discomfort. Experimental studies have shown that ginger protects the liver against the toxic effects of alcohol, country liquor, acetaminophen, and heavy metals (Haniadka et al., 2013).

Our previous study has determined the protective effect of ginger against SMB-induced testicular oxidative stress (Afkhami Fathabad et al., 2017).

The daily intake of sulfites through foods and beverages may exceed the acceptable daily intake value (0.7 mg/kg body weight) (Lien et al., 2016). Because consequences of dietary exposure to sulfites are not fully characterized, it is necessary to evaluate how the health risk associated with that can be reduced. There are no published reports in the literature about the protective effect of ginger extract against oxidative stress induced by SMB in the blood of rats. On the basis of these considerations, this study was designed to evaluate whether changes in serum biochemical and oxidative stress markers induced by SMB ingestion could be treated with ginger.

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## 2. Materials and Methods

### *2.1. Drug and Ginger Extract Preparation*

Sodium metabisulfite was obtained from Sigma (EC No: 231-673-0, CAS. No: 7681-57-4) and dissolved in distilled water (260 mg/ml). Dried ginger rhizomes were purchased from Arsanjan grocery and powdered by grinder. 200 g of the powder was soaked in 1 litre of 50% ethanol for 72 hours and extracted by percolation several times until complete exhaustion. The solvent was concentrated using rotavapor device connected to a vacuum pump. 20 g of the concentrate was obtained. Previous studies using HPLC identified the major constituents of ginger including: [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol, [8]-shogaol, [10]-shogaol and [6]-paradol, and [1]-dehydrogingerdione (Shao et al., 2010).

### *2.2. Animals*

Twenty-four male Wistar rats weighing 200–250 g were maintained at 12 h light–dark cycles and a constant temperature of 23±1˚C at all times. The rats were fed with a standard rodent pellet diet and drinking water ad libitum. All experimental protocols conducted on rats were performed in accordance with the standards established by the Animal Ethics Committee at the Islamic Azad University. Rats were divided into four groups of 6 animals each: Group 1 (control): rats recieved distilled water (1 ml/kg); Group 2 (S260): rats treated with SMB (260 mg/kg); Group 3 (Z500): rats treated with ginger (500 mg/kg); Group 4 (SZ), rats treated with SMB + ginger at the same previous dose. The doses of SMB and ginger were prescribed according to the previous studies (Morakinyo et al. 2010, Rezaee et al. 2016). Ginger and SMB were given by gavage via oral cannula and lasted for a period of 28 days.

### *2.3. Biochemical Evaluation*

At the end of the experimental period, rats were anesthetized using diethyl ether and the blood samples were collected by cardiac puncture. The samples were allowed to coagulate for 30 min at room temperature and centrifuged at 3000 rpm for 10 min. The supernatant serum was quickly removed and kept at –20 ˚C for further analysis.

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) , total and direct bilirubin, creatinine, BUN, and total protein values were checked using the standard diagnostic test kits (Pars Azma Co., Iran).

### *2.4. Serum Antioxidant Enzyme Activities*

(GSH) contents with 5-5'-dithiobis, 2-nitrobenzoic acid (DTNB) was measured and followed by a standard Ellman's method (Ellman, 1959). The absorbance of the reaction products was observed after 5 min at 412 nm. The glutathione peroxidase (GPx) activity of serum samples was measured by continuous monitoring of the regeneration of GSH from oxidized glutathione (G-S-S-G) upon the action of glutathione reductase (GR) and NADPH according to the method of Fecondo and Augusteyn (Fecondo and Augusteyn, 1983). The activity of GR was measured spectrophotometrically with a Randox laboratory kit at 340 nm and 37°C using the method described by Carlberg and Mannervik (Carlberg and Mannervik, 1985). Catalase (CAT) was assayed spectrophotometrically by monitoring the decomposition of $H_2O_2$ using the procedure of Aebi (Aebi, 1984). The activity of SOD was assayed according to Winterbourn et al. (1975) and is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium by superoxide (Winterbourn et al., 1975).

### *2.5. Measurement of Lipid Peroxidation*

The level of serum malondialdehyde (MDA), a product of lipid peroxidation, was determined using thiobarbituric acid (TBA) method. MDA reacts with TBA and produces a pink colored complex which has the maximum absorbance at 532 nm. The results were expressed as nmol/ml (Mihara and Uchiyama, 1978).

### *2.6. Statistical Analysis*

All data are presented as mean ± standard error of mean (SEM) and analyzed using SPSS (Version 18; SPSS Inc., Chicago, USA). Data was analyzed using one-way ANOVA and a post hoc Tukey test. Statistical significance was accepted when P < 0.05.

## 3. Results

### *3.1. .Evaluation of Liver and Kidney Function Markers*

The effects of SMB and ginger on serum biomarkers of liver and kidney function including SGOT, SGPT, ALP, TP, and billirubin (as liver function test) and BUN and creatinine (as kidney function test) were examined at the end of treatment period (Table 1). Sulfite treatment for 28 days caused a significant increase in SGOT (P = 0.001), SGPT (P = 0.008), ALP (P = 0.049), and a significant decrease in total protein (P = 0.02). BUN and creatinine levels were increased non-significantly in the SMB treated group. The levels of examined serum biomarkers in the rats fed ginger with SMB did not show any significant difference compared to control group. All measured values in the control and ginger treated groups were almost the same.

**Table1**. Effect of ginger and sodium metabisulfite treatment for 28 days on serum biochemical parameters.

| Group | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | TP (g/dl) | BUN (mg/dl) | Cr (mg/dl) | T. Bili (mg/dl) | D. Bili (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| Cont | 182.5±18.7 | 111.5±9.5 | 456±30.9 | 6.7±0.2 | 23.6±0.4 | 0.86±0.02 | 0.07±0.007 | 0.02±0.01 |
| Z500 | 180.5±9.7 | 107±4.3 | 364.6±43.6 | 6.6±0.1 | 22.5±2.1 | 0.9±0.03 | 0.1±0.001 | 0.04±0.1 |
| S260 | 266.6±2.6** | 148.3±3.8** | 683.8±78.1* | 5.7±0.2* | 26.1±1.2 | 1.17±0.2 | 0.1±0.001 | 0.03±0.01 |
| SZ | 202.1±13.7## | 117.6±8.8# | 509.6±63.7 | 6.2±0.1 | 19.3±1.2# | 0.82±0.01 | 0.06±0.008# | 0.008±0.01 |

Each value indicates the mean ± SEM. Z500, administration of 500 mg/kg/day ginger; S260, administration of 260 mg/kg/day sodium metabisulfite; SZ, coadministration of sodium metabisulfite and ginger at the same dose. *P < 0.05, **P < 0.01 vs. control group; #P < 0.05, ##P < 0.01 vs. S260.

### *3.2. Evaluation of Serum Antioxidant Enzyme Activities*

As observed in Table 2, administration of SMB to rats resulted in significant decrease in serum concentrations of GSH ($P = 0.001$) and activities of GPx, GR ($P < 0.001$), and catalase ($P = 0.03$) when compared with the control group. Co-administration of SMB with ginger extract resulted in significant increase in the GPx and GR activities when compared to the SMB treated rats. No statistically significant difference was observed in GSH level and catalase activity in rats treated with SMB + ginger and control group. No difference was observed between control and ginger treated rats.

**Table2.** Effect of ginger and sodium metabisulfite treatment for 28 days on the serum antioxidant enzyme activities.

| Groups | SOD (u/ml) | GPx (u/ml) | GR (u/ml) | CAT (u/ml) | GSH (µmol/ml) |
|---|---|---|---|---|---|
| Control | 6.4±0.74 | 17.1±1 | 3.4±0.2 | 19.1±1.9 | 0.76±0.08 |
| Z500 | 6.1±1.7 | 24.3±1.17 | 6.7±0.08 | 13.1±1.5 | 0.63±0.04 |
| S260 | 5.2±1 | $1.17±0.26^{***}$ | $1.46±0.04^{***}$ | $8.1±3.1^{*}$ | $0.36±0.06^{**}$ |
| SZ | 6.5±1.1 | $7.93±0.45^{***\#\#\#}$ | $2.6±0.04^{**\#\#}$ | 9.8±3.1 | 0.59±0.03 |

Each value indicates the mean ± SEM. Z500, administration of 500 mg/kg/day ginger; S260, administration of 260 mg/kg/day sodium metabisulfite; SZ, coadministration of sodium metabisulfite and ginger. SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase; GSH, reduced glutathione. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. S260.

### *3.3. Evaluation of Serum Lipid Peroxidation*

Serum MDA levels (means ± SEM) detected in SMB treated rats (33.03 ± 1 nmol/L) were significantly higher than those detected in rats treated with ginger (23.7 ± 2.1 nmol/L) and control (18.9 ± 2.1 nmol/L). Though not significant, MDA levels were observed to decrease in rats treated with SMB + ginger (27.7 ± 0.5 nmol/L) (Fig. 1).

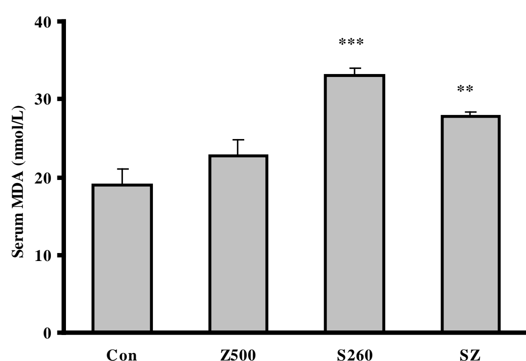


**Figure 1.** Effect of sodium metabisulfite on serum malondialdehyde (MDA) in rats after 28 days treatment. Values are expressed as mean±SE for six rats in each group. Con, control group; Z500, rats treated with ginger (500 mg/kg), S260, rats treated with sodium metabisulfite (260mg/kg); SZ, rats treated with sulfite and ginger. Asterisk, significantly different from the control group. $^{**}P < 0.01$, $^{***}P < 0.001$.

## 4. Discussion

In the present study, the serum biomarkers of oxidative stress and renal and hepatic injuries subsequent to exposing rats to SMB at 260 mg/kg dose were evaluated. The results show that SMB ingestion increased serum levels of MDA, accompanied by significant reduction of GPx, GR, CAT activities and GSH concentration. The present data also confirmed that administration of SMB for 28 days caused liver injury as evidenced by elevation of SGOT, SGPT, ALP, and reduction of total protein. The modification in the values of the investigated parameters confirms the hepatoprotective effect and antioxidant activity of ginger.

The main mechanism by which sulfite mediates its toxic effects is through oxidative stress due to an increased production of sulfur- and oxygen-centered free radicals. ROS overproduction and ATP depletion have been shown in neurons and human fetal liver cells due to sulfite toxicity (Zhang et al. 2004). Lipid peroxidation was induced in the kidney and liver of rats treated orally with SMB (Elmas et al. 2005). The observed increase in serum MDA levels, as a byproduct of lipid peroxidation, confirms previous observations of sulfite-induced lipid oxidation as a well-established mechanism of cellular injury (Shekarforoush et al. 2018). Sulfite-induced hepatotoxicity is associated with the rapid disappearance of oxidized glutathione (GSSG), followed by the slow depletion of reduced glutathione that potentially diminishes antioxidant defense (Niknahad and O'Brien 2008). Decreased serum antioxidant enzymes activity and GSH content as a result of oxidative stress can reduce the protection against free radicals and lipid peroxidation. The results of an experimental study showed that ingested sulfite by inducing hepatocyte necrosis may cause liver dysfunction (Bai et al. 2013). Hepatotoxicity linked to oxidant stress is reflected by an increase in the levels of hepatic enzymes (Contreras-Zentella and Hernandez-Munoz 2016). Significant increase in the SGOT, SGPT and ALP activities in the SMB treated group could be taken as an index of liver damage. The findings of this study indicated that ginger prevented hepatic enzyme changes in rats. Ginger significantly decreased the serum levels of transaminases towards the respective normal values and increased the activities of GPx and GR compared to SMB ingestion alone. These alterations indicate that the ginger is somewhat able to stabilize plasma membranes and repair hepatic tissue damage caused by SMB. These results are consistent with previous reports indicating that ginger exerts antioxidative effect by decreasing lipid peroxidation and maintaining normal levels of antioxidant enzymes (Ahmed et al. 2000, Mashhadi et al. 2013). Increased serum GSH level was reported in ginger fed rats (Ahmed et al. 2000). Hepatoprotective effect of ginger extract has been demonstrated in earlier studies (Atta et al. 2010, Yemitan and Izegbu 2006). The protective effect of ginger may be due to prevention of the decline of hepatic antioxidant status or its direct radical scavenging capacity (Ajith et al. 2007). Recent studies revealed that ginger active components 6-shogaol (Peng et al. 2015) and 6-dehydrogingerdione (Yao et al. 2014) are potent activators of the transcription factor Nrf2 to boost the cellular antioxidant enzymes and GSH.

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The previous reported data indicated that SMB, when given at a dose of 520 mg/kg/day, results in increased lipid peroxidation in the kidney (Elmas et al. 2005). Contrary to the finding of abnormal elevation of liver function tests, the sulfite at a dose of 260 mg/kg/day did not significantly increase the serum level of BUN and creatinine. The finding suggests that hepatocytes may be more sensitive to sulftes. On the other hand, because of renal reserve, the serum creatinine level may not rise until 50% of kidney function has been lost (Ronco 2013).

Although this study provides important findings about protective effect of ginger against SMB, the lack of histology was a limitation of our study. Future studies should include histology to confirm the effects of SMB and ginger. In addition, we recommend evaluating the effect of the active gradients, such as 6-shogaol and 6-dehydrogingerdione, as an important line of study.

## 5. Conclusion

The present results show that sodium metabisulfite (260 mg/kg) induces oxidative stress through decreased serum antioxidant enzyme activities and GSH and increased MDA level.

The observed oxidative effect of sulfite correlates with elevated liver enzymes suggesting that increased ingestion of sulfite may cause damage to the hepatocytes. Increased GPx and GR activity and decreased serum transaminases by co-administration of ginger with sulfites suggest that ginger may partially reduce the observed oxidative damage in the rats after exposure to sulfite.

## 6. Conflict of Interests

The authors declare that there is no conflict of interests in relation to this work.

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