

Study on the pharomic character of an insulin-mimetic small molecular compound of Vanadyl Trehalose

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SUMMARY

To investigate the effect of vanadyl trehalose (VT) on oxidative stress and reduced glutathione/glutathione-S-transferase (GSH/GSTs) pathway gene expression in mouse gastrointestinal tract, as well as the protective effects of vitamin C (VC) and reduced glutathione (GSH). Thirty male Kunming mice were randomly divided into five groups: control group (group A), VT Group (group B), VC + VT Group (group C), GSH + VT Group (group D) and VC + GSH + VT Group (group E). The content of reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) activity and the expressions of glutamate-cysteine ligase catalytic subunit (*GCLC*), glutathione synthetase (*GSS*), regulated through glutathione

reductase (*GSR*) and glutathione-S-transferase pi (*GSTpi*) in stomach and duodenum in vanadyl trehalose treated group were lower than those in group A ($P<0.05$). The C, D, E group can significantly improve the above indicators, but those only in the stomach in E group reached the level of the control group. Vanadyl trehalose (VT) was able to cause oxidative stress damage to the gastrointestinal tract of mice, which affects GSH content and GSH-Px activity and interferes with the normal expression of GSH/GSTs pathway. Exogenous vitamin C, reduced glutathione and the combination of the two could play a specific role in antioxidant protection and reduce the toxicity of vanadyl trehalose.

KEY WORDS: Vanadyl trehalose (VT); Oxidative Stress; GSH/GSTs; Vitamin C;

Glutathione

1. Introduction

In recent years, with the improvement of people's living standards, the incidence of diabetes is increasing every year (Canivell and Gomis, 2012, Radosinska *et al.* 2015). Diabetes mellitus is a chronic metabolic disease caused by insufficient insulin secretion or decreased insulin sensitivity in the body (Gavin *et al.* 1997; Whiting *et al.* 2013, Xiaodan *et al.* 2019). Vanadium can regulate the metabolism of sugar to a certain extent and anti-diabetes function (Srivastava and Mehdi, 2005, Jiang *et al.* 2016, Wilk *et al.* 2017). However, vanadium compounds, while resisting diabetes, often lead to some toxic effects (Jiang *et al.* 2017), mainly the digestive tract, such as diarrhea, dehydration, weight loss, etc. (Imura *et al.* 2013). It is reported that the mechanism of the toxicity of

vanadium may be related to the free radical accumulation, the decrease of antioxidant enzyme activity such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) etc. and the enhancement of oxidative damage. (Liu *et al.* 2012, Lee *et al.* 2012).

Glutathione (GSH) is an important antioxidant *in vivo*, and its content is mainly regulated through glutathione reductase (GSR), glutathione synthetase (GSS), glutamic acid cysteine junction enzyme (γ -GCL) and some glutathione -S- transferase enzyme (GSTs) (Lu, 2013). These enzymes together constitute a GSH/GSTs pathway (Kalinina *et al.* 2014). Vitamin C is a reducing agent for chemical reactions inside and outside the cell, and a water-soluble low molecular antioxidant (Fiammetta *et al.* 2017).

Although toxic effects have been mainly reported from inorganic forms, organic vanadium compounds have been associated with therapeutic effects. It may be due to the increased lipophilicity and gastrointestinal absorption (Wang *et al.* 2001). Thus, organic vanadium compounds have become a highlight. Vanadyl trehalose (VT) was originally a new type of organic vanadium compound synthesized by Barrio *et al.* (Barrio *et al.* 2003). We used a similar method to synthesize VT, found that it could regulate the blood glucose level of diabetic mice and alleviate the symptoms of diabetes such as polydipsia, polyphagia, polyuria and body mass loss (Jiang *et al.* 2016; Jiang *et al.* 2018). Further studies have found that the main toxic target organ of trehalose in Kunming mice is the stomach and duodenum (Jiang *et al.* 2017, Jiang *et al.* 2018).

“This study was performed to observe the effects of vanadyl trehalose (VT) on oxidative stress and (GSH/GSTs) pathways gene expression in the stomach and

duodenum of experimental mice and to explore the detoxification effect of exogenously added vitamin C (VC) and reduced glutathione (GSH).”

2. Materials and Methods

2.1. Laboratory Animals

Thirty male Kunming mice of SPF grade, five weeks old, were purchased from the Center of Chinese Academy of Military Medical Sciences (animal license number SCXK-2007 -005). The animal room maintained temperature (20 ± 2) °C, humidity (50 ± 5) %, and the light and dark cycle was 12h:12h, without strong acousto-optic stimulation. All animals were kept in the environment for 1 week, during which they were given free access to standard feed and open access to tap water.

2.2. Reagents

The main reagent vanadium sulphate was purchased from Tianjin Alfa Aesar Chemical Co., Ltd.; trehalose was purchased from Tianjin SMS Co., Ltd.; anhydrous ethanol was purchased from Tianjin Chemical Reagent Supply and Marketing Company; GSH was purchased from Beijing Solabao Company; VC was purchased from US Sigma; GSH and GSH-Px activity assay kits were purchased from Nanjing Institute of Bioengineering; RNAiso Plus was purchased from TaKaRa; FastKing cDNA first strand synthesis kit and superReal PreMix plus SYBR Green purchased from Beijing Tiangen Biochemical Technology Co., Ltd.; all the primers were synthesized by Beijing Aoke Dingsheng Biotechnology Co., Ltd.

2.3. Synthesis of trehalose vanadate

The synthesis of trehalose vanadate is in accordance with the method of Barrio *et al.* 2003. To 10 ml of trehalose solution (0.4 mol/l), vanadium sulphate solution (0.1 mol/l) was added dropwise, and then the pH of the mixed solution was adjusted using NaOH solution (2 mol/l). The brown mixed solution was sealed at room temperature for 12 to 24 hours, anhydrous ethanol was added, and a solid was gradually precipitated, later the solid was collected by filtration and washed with water-free ethanol. The product was dried over NaOH solids and stored in a desiccator. Various studies have carried out using standard concentration of VC and GSH. The concentrations of vitamin C and GSH was measured according to Su *et al.*, 2014, Zhao *et al.* 2018 studies. VT, VC and GSH were dissolved in distilled water and used on the same day.

2.4. Experimental grouping and drug administration

Thirty male Kunming mice were randomly divided into 5 groups and treated as follows: Group A: gavage of equal volume of normal saline; Group B: gavage of 125 mg / (kg·d) VT; Group C: After gavage of 150 mg / (kg·d) VC 1 hour, then gavage of 125mg / (kg·d) VT; Group D: After gavage of 150 mg / (kg·d) GSH for 1 h, then gavage of 125mg / (kg·d) VT; Group E: After gavage of 150 mg / (kg·d) VC+150mg / (kg·d) GSH 1 hour, then gavage of 125mg / (kg·d) VT. Each group was administered continuously for 15 days.

2.5. Sampling and sample preparation after the last administration

After the last administration, the mice in each group fasted for 24 hours. The mice were sacrificed by cervical dislocation and quickly dissected. The stomach and duodenum were rinsed 2 to 3 times in pre-cooled physiological saline, dry with filter paper. Each sample was prepared in duplicate, one part was placed in an EP tube, and stored in a refrigerator at -20 °C for storage; the other part was placed in a cryotube and frozen in liquid nitrogen, and stored in a refrigerator at -80 °C for use. Preparation of tissue homogenate: weigh the stomach and duodenum tissue (0.2~1g) stored at -20 °C and add 9 times pre-cooled homogenate medium (normal saline) at 1:9 (g/ml). In the mortar, the homogenate was prepared under ice bath conditions, centrifuged at 3000 rpm for 10 min, and the supernatant was frozen in a refrigerator at 4°C or frozen in a freezer at -20 °C for use.

2.6. GSH content and GSH-Px activity determination

Reduced glutathione (GSH) content was determined by using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) method. GSH reacts with DTNB yield a colored product 5-thio-2-nitrobenzoic acid (TNB). The rate of formation of TNB, measured at 405 nm, is proportional to the concentrations of GSH in the sample. It's a reliable method to measure reduced cysteines and other free sulfhydryls in solution. (Rahman *et al.* 2006). GSH-Px activity was measured by GSH oxidation. The determination and calculation of each of the above indicators were carried out in strict accordance with the procedure of the kit instructions.

2.7. Real-time fluorescence quantitative PCR detection

The total RNA of the stomach and duodenum tissues was extracted with reference to the procedure of the RNAiso Plus instruction. Total RNA concentration and purity were measured by a micro-ultraviolet analyzer (Nano Drop 2000), and RNA integrity was analyzed by agarose gel electrophoresis. The RNA extract was stored at -80 °C until use. The total RNA of each sample was reverse-transcribed into cDNA using the Fast King cDNA first-strand synthesis kit, and used as a template according to Super Real Pre Mix Plus SYBR Green instruction, and the relevant reagents and primers were added to carry out PCR amplification in a final reaction volume of 20 µl. The primer sequences used are shown in Table 1. Reaction conditions: pre-denaturation at 95 °C for 15 min; denaturation at 95 °C for 10 s, annealing at 57 °C for 20 s, extension at 72 °C for 20 s, 40 cycles; detection signal at 72 °C. The Ct value was calculated by using CFX96 PCR thermal cycler analysis software according to the formula $2^{-\Delta\Delta Ct}$ and used for statistical analysis.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 22.0 software. Data were expressed as mean \pm standard deviation ($\bar{x} \pm s$). Data between groups were compared by one-way analysis of variance (ANOVA) and LSD, the difference with $P < 0.05$ was reputed as statistically significant. F is one of the most common statistical tools for hypothesis testing for the analysis of variance (ANOVA). A statistical F Test use to compare

two variances, S_1 and S_2 , by dividing them. Variances are a measure of dispersion, or how far the data are scattered from the mean. The result is always a positive number (because variances are always positive). The equation for comparing two variances with the F Test is: $F = S_1^2 / S_2^2$. If $F > F_{0.05}$ or $F_{0.01}$, indicating that the differences are significant.

3. Results

3.1. Comparison of GSH content in stomach and duodenum from different groups of mice

After 15 days of intervention in the 5 groups of mice, the GSH content in the stomach tissue ranged from 27.4 to 34.46 $\mu\text{mol/g}$ protein and in the duodenum ranged from 7.4 to 56.46 $\mu\text{mol/g}$ protein (Table 2). The GSH content was comparatively higher in duodenum than that in stomach. Statistically, the GSH values in stomach was significant ($F = 28.25$, $P < 0.01$), The content of GSH in the stomach of VT treated mice was significantly lower than in the control group ($P < 0.01$), The content of GSH in the VT-treated mice was also lower than mice in VC + VT Group (group C), GSH + VT Group (group D) and VC + GSH + VT Group (group E) (both $P < 0.01$); Mice having treatment of vitamin C, GSH and vanadyl trehalose in E group have higher contents of GSH than mice of C and D group. ($P=0.008$, $P=0.023$), The difference of GSH contents in the stomach of mice treated with vitamin C and vanadyl trehalose of C group and mice treated with GSH and vanadyl trehalose of D group was statistically not significant. ($P = 0.538$), GSH contents of mice in control group have no significant

difference than mice of E group treated with vitamin C, GSH and vanadyl trehalose. (group A) ($P = 0.405$, Table 2). There was a significant difference in GSH content between the duodenum of the 5 groups ($F = 76.17$, $P < 0.01$). Mice treated with vanadyl trehalose has significantly lower GSH contents in duodenum than mice of control group ($P < 0.01$), and the C, D, E groups were significantly higher than the mice having VT treatment in group B (both $P < 0.01$). There was no significant difference between the mice of C and D group. ($P = 0.154$); the VC + GSH + VT Group (group E) was significantly higher in groups VC + VT Group (group C) and GSH + VT Group (group D) ($P = 0.003$, $P = 0.0047$), but still significant lower than group A ($P = 0.035$, Table 2).

3.2. Comparison of GSH-Px activity in stomach and duodenum of mice

The GSH-Px activity in the stomach tissue was high as (51.98-63.01 units/mg protein) than in the duodenum (29.94-50.11 units/mg protein) (Table 3). The GSH-Px activity was comparatively higher in control group (group A) and VC + GSH + VT Group (group E). The lowest GSH-Px activity values of duodenum was found in mice treated with vanadyl trehalose (group B). Overall, the activity of GSH-Px in the five groups was significantly different ($F = 9.44$, $P < 0.01$) (Table 3). Compared with the control group (group A), the GSH-Px activity of mice treated with vanadyl trehalose in B group decreased significantly ($P < 0.01$), mice having vanadyl trehalose treatment of B group has lower GSH-Px activity than mice of VC + VT Group (group C), GSH + VT Group (group D) and VC + GSH + VT Group (group E) (both $P < 0.01$). There was no significant difference between mice having treatment of Vitamin C and vanadyl

trehalose of C group and mice having treatment of GSH and vanadyl trehalose of D group ($P = 0.923$). Mice of group E with VC, GSH and VT treatment was significantly higher than mice having treatment of VC + VT Group (group C) and GSH + VT Group (group D) ($P=0.077$, $P=0.043$). There was no significant difference between GSH-Px activity in mice of VC+ GSH + VT Group (group E) and mice of control group (group A) ($P=0.77$, Table 3). There was a significant difference of GSH-Px activity in the duodenum between the 5 groups ($F=340.39$, $P < 0.01$). The GSH-Px activity of the duodenum in VT treated mice in group B was significantly lower than that mice in control group (group A) ($P < 0.01$); Compared with VT Group (group B), the VC + VT Group (group C), GSH + VT Group (group D) and mice having treatment of VC + GSH + VT (group E) were significantly improved (both $P < 0.01$), and group VC + GSH + VT Group (group E) was significantly higher than VC + VT Group (group C) and GSH + VT Group (group D) ($P=0.003$, $P=0.018$), but the difference between groups VC + VT Group (group C) and GSH + VT Group (group D) was not statistically significant ($P=0.325$); Mice having treatment of vanadyl trehalose (group B) was still lower than control group (group A), and the difference was statistically significant ($P=0.008$, Table 3).

3.3. Changes of mRNA expression of GSH/GSTs pathway-related genes in stomach of mice

After 15 days of continuous treatment, the expression of each gene in the gastric tissue of the 5 groups was statistically significant (*GCLC*: $F = 32.94$, $P < 0.01$; *GSS*: $F = 22.73$, $P < 0.01$; *GSR*: $F = 20.22$, $P < 0.01$; pi-glutathione S-transferase (*GSTpi*): $F =$

27. 39, $P < 0.01$); The expressions of *GCLC*, *GSS*, *GSR* and *GSTpi* in gastric tissue of VT Group (group B) mice were significantly lower than those in mice of control group (group A) (both $P < 0.01$). The expressions of *GCLC*, *GSS* and *GSR* in VC + VT Group (group C), GSH + VT Group (group D) and VC + GSH + VT Group (group E) were significantly higher than those mice treated with vanadyl trehalose in group B (both $P < 0.05$). The expression of *GCLC*, *GSS* and *GSR* in mice group E having treatment of Vitamin C, GSH and vanadyl trehalose was significantly higher than mice in VC + VT Group (group C) and GSH + VT Group (group D) (both $P < 0.05$), but no significant difference with control group (group A), and the difference between VT Group (group C) and GSH + VT Group (group D) is not obvious (both $P > 0.05$); the expression of *GSTpi* has no statistically significant difference among VC + VT Group (group C), GSH + VT Group (group D) and group E ($P > 0.05$, Fig. 1).

3.4. Changes of mRNA expression of GSH/GSTs pathway-related genes in duodenum of mice

The expression of each gene in the duodenum of the 5 groups was statistically significant (*GCLC*: $F = 126.74$, $P < 0.01$; *GSS*: $F = 21.86$, $P < 0.01$; *GSR*: $F = 26.41$, $P < 0.01$; *GSTpi*: $F = 41.05$, $P < 0.01$). Compared with control group (group A), the *GCLC*, *GSS*, *GSR* and *GSTpi* expression in the mice duodenum of VT Group (group B) were all significantly down-regulated (both $P < 0.01$), and the mice in VC + VT Group (group C), GSH + VT Group (group D) and VC + GSH + VT Group (group E) were significantly higher than those of the mice having vanadyl trehalose treatment (group

B) (both $P < 0.05$). Compared with the VC + GSH + VT Group (group E) was significantly increased (both $P < 0.05$), but only *GSR* expression returned to the control group (group A) ($P = 0.954$). There were no significant differences in the expressions of *GCLC*, *GSS* and *GSTpi* between mice of VC + VT Group (group C) and GSH + VT Group (group D) (all $P > 0.05$), but the expression of *GSR* in mice with vitamin C and vanadyl trehalose treatment Group (group C) was significantly higher than that in GSH + VT Group (group D) ($P = 0.035$); there was no significant difference in *GSTpi* expression among the VC + VT Group (group C), GSH + VT Group (group D) and VC+ GSH + VT Group (group E) are all significantly lower than the control group (group A) (all $P < 0.05$, Fig. 2).

4. Discussion

Oxidative stress refers to the body suffering a variety of harmful stimuli, the highly active molecules such as reactive oxygen species (ROS) excessive production, the body cannot be removed in time, oxidation system and antioxidant system imbalance, resulting in tissue damage (Gyuraszova *et al.* 2018, Trebaticky *et al.* 2019). ROS include superoxide anion ($\cdot\text{O}_2^-$), hydroxyl radical ($\cdot\text{OH}$) and hydrogen peroxide (H_2O_2) (Wiecek *et al.* 2015, Linhartova *et al.* 2016). Under physiological conditions, vanadium exists mainly in the form of +5 and +4 valence states, and the form transition frequently accompanied the ROS production. V^{4+} generates V^{5+} through the Fenton reaction and produces a large amount of $\cdot\text{OH}$. After the vanadium compound stimulates the cells,

the intracellular P47phox protein is phosphorylated, thereby activating NADPH oxidase and producing a large amount of O₂⁻ in the cells (Lushchak, 2014). There are two types of antioxidant systems in the body: one is the enzyme antioxidant system, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), etc.; and the other is non-enzymatic antioxidant system, including vitamin C, vitamin E, GSH, carotenoids and selenium (Se) (Patrik *et al.* 2017).

In this study, after 15 days of continuous intragastric administration, the results showed that the GSH content and GSH-Px activity in the stomach and duodenum of the VT group were lower than those of the control group. The main reasons may be: 1) GSH is oxidized and consumed during the conversion of pentavalent vanadium to tetravalent vanadium; 2) vanadium and GSH have high affinity, which leads to direct binding; 3) the GSH oxidized by vanadium induced ROS leads a decrease in the level of GSH in the body cells. Lack of or depletion of GSH in the body can aggravate the toxic effects of various chemicals and increase oxidative damage (Nemeti *et al.* 2002). Mice fed with VT reduced the activity of glutathione peroxidase (GSH-Px), probably because its vanadium ion has a strong affinity with -SH, and the binding of -SH to the active center of GSH-Px leads to a decrease in viability. In addition, it may be related to the fact that vanadium is easily combined with the GSH-Px substrate GSH to reduce the GSH-Px activity.

Infusion of VT impairs the gastrointestinal antioxidant system of mice and causes oxidative stress in the body. Folarin *et al.* 2017 found that vanadium caused a decrease in GSH content and GSH-Px activity in mouse brain. It has also been reported in the literature that vanadium can reduce the GSH content of rat hepatocytes (Hosseini *et al.* 2013). The results of this study showed that exogenous supplementation of VC and GSH can increase the GSH content and GSH-Px activity in the stomach and duodenum of mice, but only when the two are used together, the GSH content and GSH-Px activity in the stomach can be restored to the control level, indicating that the effect of VT on duodenal GSH content and GSH-Px activity was more evident than that of gastric tissue. Exogenous GSH supplementation can directly increase the GSH content in the body, help to scavenge free radicals, and produce cross-linking complexes with electrophilic complexes under the action of non-enzymatic or glutathione thiol transferase (GST) to form sulfur etherase excreted *in vitro*, increasing cell detoxification function, accelerating the excretion rate of toxic by-products, and reducing the toxicity of vanadium. Wang *et al.* 2015 reported that exogenous GSH can accelerate the excretion of arsenic in mice and reduce the oxidative stress induced by arsenic, thus reducing the toxicity of arsenic. Exogenous supplementation of VC can increase collagen synthesis and repair, protect mucosal cells, maintain the integrity of the structure and function of the gastrointestinal tract, and rapidly react with $\cdot\text{O}_2^-$, H_2O_2 and $\cdot\text{OH}$ to form semi-dehydroascorbic acid to eliminate or reduce these free radicals thus prevent or reduce oxidative damage in the cells. It has also been reported that VC can increase the

antioxidant capacity of intestinal epithelial cells and reduce their oxidative damage (Liu *et al.* 2012).

GSH/GSTs are essential antioxidant and detoxification systems for animals. GSH synthesis (*GCLC*, *GSS*, *GSR*) and catabolism-related enzyme genes (*GSTpis*) constitute an intact GSH/GSTs functional system and a relatively complete pathway (Kalinina *et al.* 2014). *In vivo*, reduced GSH is synthesized by γ -GCL and *GSS* with an amino acid as substrate. Among them, γ -GCL is a restriction enzyme, *GCLC* is the catalytic subunit of the enzyme; meanwhile, it can also be under the action of *GSR* and NADPH reduction of oxidized glutathione (GSSG) to GSH (Lu, 2013). The results of this study showed that the expression of *GCLC*, *GSS* and *GSR* genes in the stomach and duodenum were down-regulated in the VT-administered group compared with the control group, indicating that VT has certain toxicity to the stomach and duodenum that caused dysfunction, which interferes with the normal expression of related genes. Exogenous supplementation of VC and GSH and their combination can increase the mRNA expression of *GCLC*, *GSS* and *GSR* genes, and only the expression of gastric tissue in the combination group can be restored to the control level, which is related to the GSH content. The effects of GSH-Px activity are consistent. It is speculated that exogenous addition of VC and GSH may increase the antioxidant capacity in the body, protect the function of the stomach and duodenum and reduce the influence of VT side effects on the expression of related genes. It is worth noting that the expression of *GSR* in the duodenum in supplemental VC group was significantly higher than that in the

supplemental GSH group, and the combination of the two could restore the expression to the control level. It may be that VC is more effective than GSH to increase *GSR* activity and the amount of GSH in the body, therefore the combination supplementation of VC and GSH restore cellular function to normal levels (Liu *et al.* 2012).

Glutathione S-transferases (GSTs) belong to the isoenzyme family of phase II metabolic detoxification enzymes and have the dual functions of scavenging free radicals and detoxification *in vivo*. *GSTpi* is an important member of the GSTs family, it can participate in the metabolism of a variety of internal and exogenous substances, remove harmful substances, reduce the body's oxidative damage and protect cells (Schnekenburger *et al.* 2014). The results in this study showed that VT was toxic to the stomach and duodenum, impairing the normal function of the cells, resulting in a decrease in *GSTpi* expression. Exogenous supplementation of VC and GSH and their combination increased *GSTpi* expression, only in the stomach tissue was comparable to the control group, indicating that VT had more significant effect on the duodenum.

The dose of VT used in the study was comparable to doses in which VT effectively reduces blood glucose levels previous research reported by Barrio *et al.*, 2003 and Jiang *et al.* 2016. The results in this study also showed that although separately supplementation of VC and GSH had improved effects on each index than the VT-administered group, but they did not reach the control level. There may be limitations in the use of VC and GSH alone or may be related to insufficient supplementation. When VC and GSH are combined supplementation, which results some indicators

return to normal levels, and the effect is better than VC and GSH alone, indicating that VC and GSH have a synergistic effect in addition to their own antioxidant effects. VC and GSH form a coupled redox couple *in vivo*, when VC is oxidized to dehydrogenated VC, the activity of GSH-dependent dehydrogenase VC reductase plays an important role in maintaining the VC physiological cycle, and VC can also reduce the oxidized glutathione (GSSH) to GSH, which increases its content and assist it to function (Gao *et al.* 2002).

5. Conclusion

Vanadyl trehalose was able to cause oxidative stress damage to the gastrointestinal tract of mice, which affects GSH content and GSH-Px activity, and interferes with the regular expression of GSH/GSTs pathway related. Exogenous vitamin C, reduced glutathione and the combination of the two could play a certain role in antioxidant protection and reduce the toxicity of vanadyl trehalose. The combination of the two is better than the use alone, and the relevant indicators in the stomach can reach the normal level.

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Conflict of Interest

None

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Figure Legends

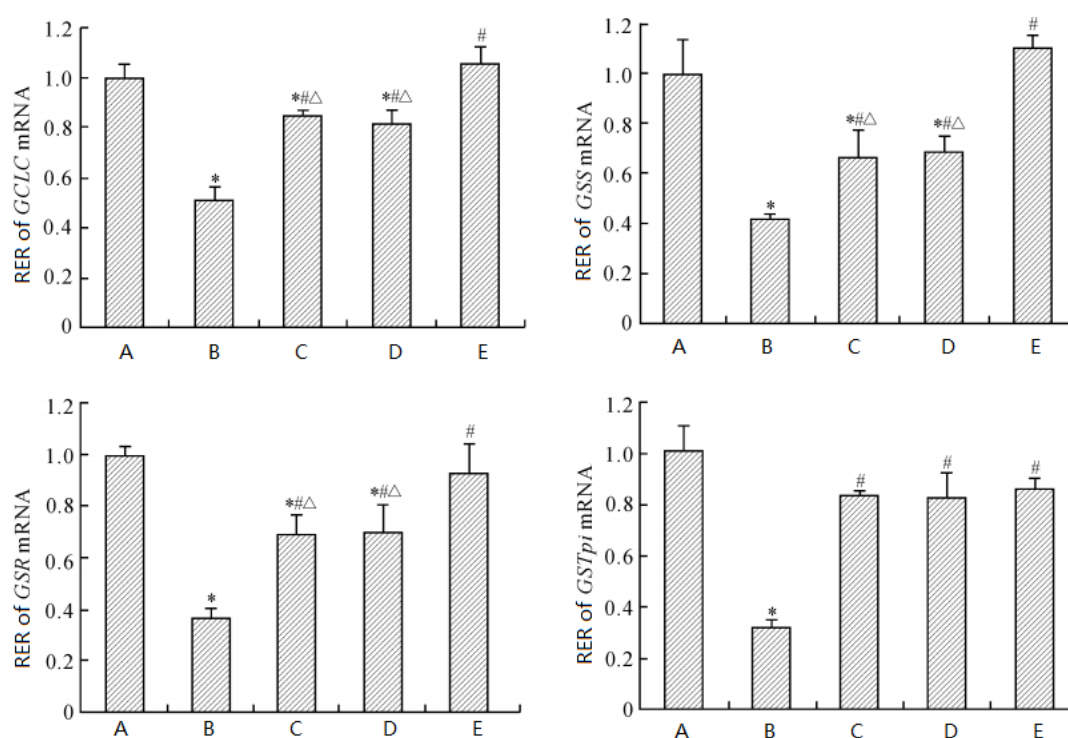


Fig. 1. The mRNA Relative expression rate(RER) of reduced glutathione/ glutathione-S-transferase GSH/GSTs pathway related genes in mice stomach tissue. The RER value of ordinate was the ratio compared with control group A; The abscissa was represented the mince groups A, B, C, D and E; The marks on each column represented the statistically significant, * was representative of significant at $p < 0.05$ level comparison with A-Group; # was representative of significant at

$p < 0.05$ level comparison with B-Group, $p < 0.05$; Δ was representative of significant at $p < 0.05$ level comparison with E-Group.

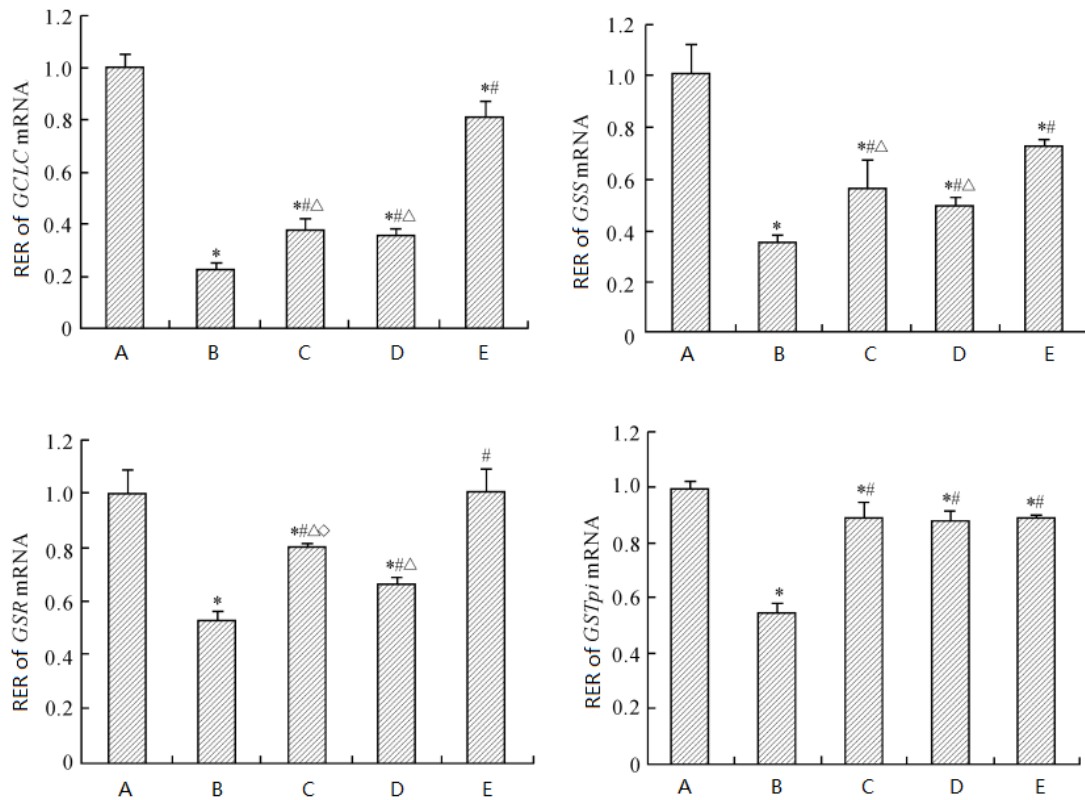


Fig. 2. The mRNA Relative expression rate (RER) of reduced glutathione/ glutathione-S-transferase (GSH/GSTs) pathway related genes in mice duodenum tissue. The RER value of ordinate was the ratio compared with control group A; The abscissa was represented the mince groups A, B, C, D and E; The marks on each column represented the statistically significant, * was representative of significant at $p < 0.05$ level comparison with A-Group; # was representative of significant at $p < 0.05$ level comparison with B-Group, $p < 0.05$; Δ was representative of significant at $p < 0.05$ level comparison with E-Group.

Table Legends

Table 1. Primers sequence for real-time PC

Gene	Sequence
<i>GCLC</i>	F:5'-CTACCACGCAGTCAAGGACC-3' R:5'-CCTCCATTCAGTAACAACACTGGAC-3'
<i>GSS</i>	F:5'-AAAGCAGGCCATAGACAGGG-3' R:5'-TGAATGGGGCATAACGTCACC-3'
<i>GSR</i>	F:5'-GCGTGAATGTTGGATGTGTACC-3' R:5'-GTTGCATAGCCGTGGATAATTTC-3'
<i>GSTPI</i>	F:5'-ATGCCACCATACACCATTGTC-3' R:5'-GGGAGCTGCCCATACAGAC-3'
<i>GAPDH</i>	F:5'-TGACCTCAACTACATGGTCTACA3' R:5'-CTTCCCATTCTCGGCCTTG-3'

Table 2. Comparison of GSH content in stomach and duodenum of mice($\bar{x} \pm s$)

Groups	Stomach ($\mu\text{mol/g protein}$)	Duodenum ($\mu\text{mol/g protein}$)
A	34.46 \pm 0.80	56.00 \pm 0.18
B	27.49 \pm 0.76 *	37.35 \pm 2.12 *
C	31.40 \pm 0.79 * Δ	48.76 \pm 1.24 * Δ
D	31.87 \pm 0.35 * Δ	50.54 \pm 0.60 * Δ
E	33.83 \pm 0.83 #	53.17 \pm 0.53 *#

The marks on right of each numeral in the table represented the statistically significant, * was representative of significant at $p < 0.05$ level comparison with A-Group; # was representative of significant at $p < 0.05$ level comparison with B-Group, $p < 0.05$; Δ was representative of significant at $p < 0.05$ level comparison with E-Group.

Table 3. Comparison of GSH-Px activity in stomach and duodenum of mice ($\bar{x} \pm s$)

Groups	Stomach (units/mg protein)	Duodenum (units/mg protein)
A	63.01±2.12	50.11±0.20
B	51.98±2.30 *	29.94±0.74 *
C	57.43±2.75 * # Δ	45.67±1.04 * # Δ
D	57.63±0.70 * # Δ	46.31±0.39 * # Δ
E	62.39±1.83 #	48.05±0.29 * #

The marks on right of each numeral in the table represented the statistically significant, * was representative of significant at $p < 0.05$ level comparison with A-Group; # was representative of significant at $p < 0.05$ level comparison with B-Group, $p < 0.05$; Δ was representative of significant at $p < 0.05$ level comparison with E-Group.