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The antioxidant activity of glucosamine hydrochloride in vitro

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Abstract—The antioxidant potency of chitin derivative-glucosamine hydrochloride was investigated employing various established in vitro systems, such as superoxide ($O_2^{\cdot -}$)/hydroxyl ($^{\cdot}OH$)-radical scavenging, reducing power, and ferrous ion chelating potency. As expected, we obtained several satisfying results, as follows: first, glucosamine hydrochloride had pronounced scavenging effect on superoxide radical. For example, the $O_2^{\cdot -}$ scavenging activity of glucosamine hydrochloride was 83.74% at 0.8 mg/mL. Second, the $^{\cdot}OH$ scavenging activity of glucosamine hydrochloride was also strong and was about 54.89% at 3.2 mg/mL. Third, the reducing power of glucosamine hydrochloride was more pronounced. The reducing power of glucosamine hydrochloride was 0.632 at 0.75 mg/mL. However, ferrous ion-chelating potency was soft. Furthermore, ferrous ion-chelating potency, the scavenging rate of radical, and the reducing power of glucosamine hydrochloride increased with their increasing concentration, and they were concentration dependent. The multiple antioxidant activity of glucosamine hydrochloride was evident as it showed considerable reducing power, superoxide/hydroxyl-radical scavenging ability. These in vitro results suggest the possibility that glucosamine hydrochloride could be effectively employed as an ingredient in health or functional food, to alleviate oxidative stress.

1. Introduction

Chitin is a polysaccharide abundant in invertebrate exoskeletons including crustacean shells and fungal cell walls.[1,2] A gigantic amount of chitin is processed as solid waste from seafood processing industry.[2,3] The natural degradation of chitin presents an important feature, not only in the global recycling of carbon and nitrogen sources, but also in the production of useful chemical reagents.[4] *N*-Acetyl-D-glucosamine (Fig. 1) or glucosamine is a monosaccharide product generated from chitin or chitosan by hydrolysis and is categorized into hexosamine and is a water-soluble substance. Glucosamine, an amino monosaccharide, is a natural component of glycoproteins found in connective tissues and gastrointestinal mucosal membranes. It is a precursor of the disaccharide unit of glycosaminoglycans which are the building-blocks of the articular cartilage, the proteoglycans.[5–9] However, the ability to synthesize glucosamine in the body declines with age. This, in turn, incapacitates the generation of proteoglycan and it is known that this incapacitation results in senile osteoarthritis.[10] Therefore, the glucosamine drew attention as a useful substance for prevention and treatment of osteoarthritis, especially glucosamine hydrochloride (Fig. 2)[11] and glucosamine sulfate.[12] As present, numerous reports have recently focused on the utility of glucosamine hydrochloride for the treatment of osteoarthritis in dogs[13,14] and horses.[15,16] It is also a therapeutic agent for inflammatory bowel diseases[17] and gastritis.[18] Glucosamine is considered a dietary supplement by the Food and Drug Administration. However, up to now,

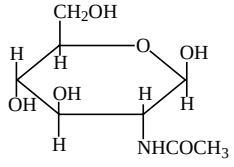

Figure 1. *N*-Acetyl-D-glucosamine.

Abbreviations: GH, glucosamine hydrochloride; NBT, nitro blue tetrazolium; PMS, phenazine methosulfate; H_2O_2, hydrogen peroxide; TBA, thiobarbituric acid; EDTA, ethylenediaminetetraacetic acid; NADH, nicotinamide adenine dinucleotide-reduced; TCA, trichloroacetic acid; DR, deoxyribose.

Keywords: Glucosamine hydrochloride; Radical scavenging effect; Reducing power; Chelating effect.

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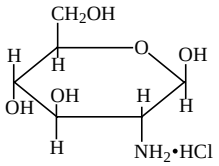

Figure 2. Glucosamine hydrochloride.

antioxidant activity of glucosamine hydrochloride has not been reported. In this paper, glucosamine hydrochloride is studied as a new antioxidant. We found that it could scavenge superoxide/hydroxyl radicals. It has pronounced reducing power. However, its ferrous ion-chelating effect is quite soft. Because it is free from side effects and non-toxic, it may be a desired food supplement as a potential antioxidant.

2. Results and discussion

2.1. Scavenging activity of superoxide radical by GH

Figure 3 show that the inhibitory effect of GH on superoxide radicals are marked and concentration related. Significant scavenging effect (72.08–83.74%) of superoxide radicals was evident at all tested concentrations of GH (0.05–0.8 mg/mL). Moreover, as shown in Figure 3, scavenging activity of superoxide radicals had reached 80.92% at 0.4 mg/mL. Compared with low molecular weight chitosan and parent chitosan, their scavenging activity for superoxide radical was 80.3% and 13% at 0.5 mg/mL, respectively.[19] Although superoxide is a relatively weak oxidant, it decomposes to form stronger reactive oxidative species, such as single oxygen and hydroxyl radicals, which initiate peroxidation of lipids.[20] In the present study, GH effectively scavenged superoxide in a concentration-dependent manner. Further, superoxides are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals.[21] These results showed GH had strong scavenging activity of superoxide radical and clearly suggested that the antioxidant activity of GH was also related to its ability to scavenge superoxide radical.

2.2. Hydroxyl-radical scavenging activity of GH

The effect of GH on oxidative damage, induced by Fe^{3+}/H_2O_2 on deoxyribose, as measured by the thiobarbituric acid method, is plotted in Figure 4. Nearly 55% inhibition was observed at the highest concentration (3.2 mg/mL). Hydroxyl-radical scavenging activity of GH was obtained in the deoxyribose system. In this system, GH exhibited a strong concentration-dependent inhibition of deoxyribose oxidation. Earlier, numerous workers[28] have employed this system to assess the biological activity of various natural plant derived biomolecules. Smith et al.[22] earlier reported that molecules that can inhibit deoxyribose degradation are those that can chelate iron ions and render them inactive or poorly active in a Fenton reaction. In the present study, in another assay system, we found that GH has considerably soft ferrous ion-chelating power, so it is impossible that the chelating effect of GH on metal ions may be responsible for the inhibition of deoxyribose oxidation. Therefore, the mechanism of GH on scavenging hydroxyl radical needs to be further researched.

2.3. Chelating effects on ferrous ions

The ferrous ion-chelating effect of GH was concentration related as shown in Figure 5. Chelating effect of GH slowly improved with the increasing concentration. At 0.1 mg/mL, ferrous ion-chelating effect of GH was about 2.53% and at 0.6 mg/mL, that of GH was 5.19%, quite-slight change occurring. The result showed that GH hardly has ferrous ion-chelating activity.

2.4. Reducing power of GH

Figure 6 depicts the reducing power of GH. The reducing power of GH correlated well with increasing concentrations. Figure 6 shows that the reducing

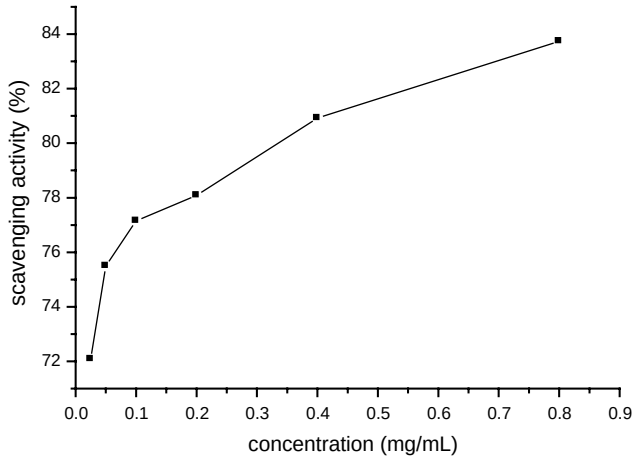

Figure 3. Scavenging effect of GH on superoxide radical. Values are means ± SD of three determinations.

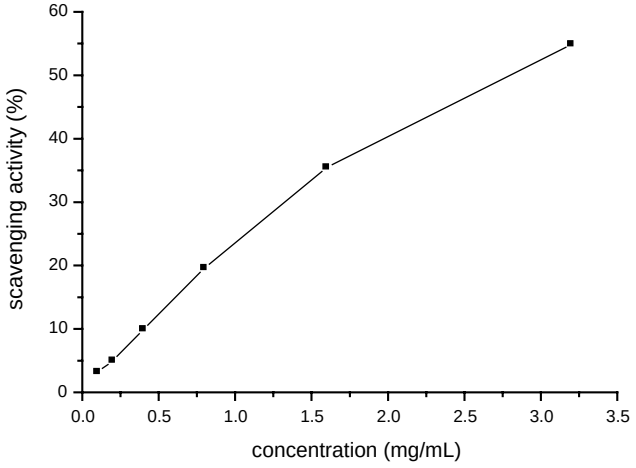

Figure 4. Inhibitory effect of GH on deoxyribose oxidative damage. Values are means ± SD of three determinations.

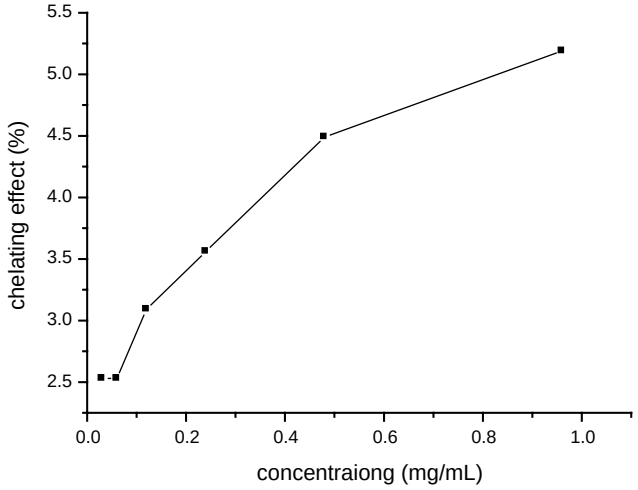

Figure 5. Chelating effect of GH on ferrous ions. Each value is expressed as the mean ± SD ($n = 3$).

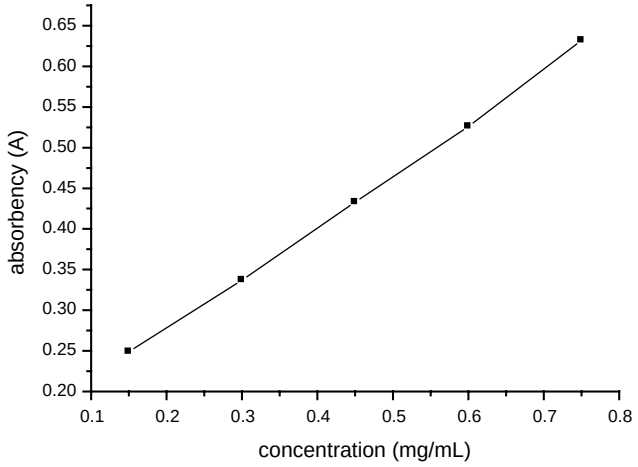

Figure 6. Reducing power of GH. Each value is expressed as mean ± SD ($n = 3$).

power increased with increasing GH concentration. Moreover, it has quite well linear relation. Jeng-Leun Mau et al.[23] reported that reducing powers were 0.80, 0.89, and 0.92 at 1.0 mg/mL for ascorbic acid, α-tocopherol, and BHA, respectively. However, as shown in Figure 6, according to linear equation: $y = kx + b$, we obtained the reducing power of GH to be 0.772 at 1.0 mg/mL. This result was similar to those of ascorbic acid, α-tocopherol, and BHA. Earlier authors[24] have observed a direct correlation between antioxidant activities and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones,[25] which have been shown to exert antioxidant action by breaking the free-radical chain by donating a hydrogen atom.[26] Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing power of GH suggested that it was likely to contribute significantly toward the observed antioxidant effect.

3. Conclusion

Except for ferrous ion-chelating potency, the multiple antioxidant activity of GH was evident as it showed considerable reducing power, superoxide/hydroxyl-radical scavenging ability. These in vitro results suggest the possibility that GH could be effectively employed as an ingredient in health or functional food, to alleviate oxidative stress.

4. Materials and methods

4.1. Chemicals

Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), ferrozine, nicotinamide adenine dinucleotide-reduced (NADH), trichloroacetic acid (TCA), deoxyribose (DR), potassium ferricyanide and ferric chloride were purchased from Sigma Chemicals Co. All other chemicals and reagents, unless otherwise specified, were not purified, dried or pretreated. Glucosamine hydrochloride (GH) was also purchased from Sigma Co.

4.2. Superoxide-radical scavenging assay

The superoxide radical scavenging ability of GH was assessed by the method of Nishikimi et al.[27] The reaction mixture, containing GH (0.05–0.8 mg/mL), PMS (30 μM), NADH (338 μM), and NBT (72 μM) in phosphate buffer (0.1 M, pH 7.4), was incubated at room temperature for 5 min and the absorbance was read at 560 nm against a blank. The capability of scavenging superoxide radical was calculated using the following equation:

$$\text{scavenging effect } (\%) = \left(1 - \frac{A_{\text{sample } 560\text{nm}}}{A_{\text{control } 560\text{nm}}}\right) \times 100.$$

4.3. Hydroxyl-radical assay

The reaction mixture, containing GH (0.1–3.2 mg/mL), was incubated with deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 μM), EDTA (100 μM), and ascorbic acid (100 μM) in potassium phosphate buffer (20 mM, pH 7.4) for 60 min at 37 °C.[28] The reaction was terminated by adding 1 mL TBA (1% w/v) and 1 mL TCA (2% w/v), and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 535 nm against reagent blank. Decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose.

4.4. Metal ion-chelating assay

The ferrous ion-chelating potency of GH was investigated according to the method of Decker and Welch,[29] wherein the Fe^{2+}-chelating ability of GH was monitored by

measuring absorbance of the ferrous iron–ferrozine complex at 562 nm. Briefly, the reaction mixture, containing GH of different concentration, $FeCl_2$ (2 mM), and ferrozine (5 mM), was adjusted to a total volume of 0.8 mL with water, shaken well, and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank. The ability of GH to chelate ferrous ion was calculated using the following equation:

$$\text{chelating effect } (\%) = (1 - A_{\text{sample 562nm}}/A_{\text{control 562nm}}) \times 100.$$

4.5. Measurement of reducing power

The reducing power of GH was quantified by the method described earlier by Yen and Chen[30] with minor modifications. Briefly, 1 mL of reaction mixture, containing different concentration of GH in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50 °C for 20 min. The reaction was terminated by TCA solution (10% w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

4.6. Statistical analysis

All data are expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the means were separated by Duncan's multiple range test. The results were processed by the computer programs: Excel and Statistica software (1999).

Acknowledgments

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