

Anti-oxidant and cytotoxic effect of collagen extracted from local buffalo

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Abstract

This study aimed to consider the DPPH radical scavenging recreation and cytotoxicity potentials of collagen extracted from buffalo. The result of antioxidant (DPPH) indicated that collagen possessed the ability to scavenge free in a concentrations dependant manner. They were (67.86±3.65, 54.44± 2.05, 47.92 ±0.53, 44.29±1.80 and 18.02±5.74%) for (200,100,50,25 and 12.5 mg/ml) respectively. the results of cytotoxicity assay match the antioxidant results, the cell viability decrease by concentrations increasing to be 40.20± 3.06, 48.64± 1.48, 61.57± 1.33, 71.14± 0.82 and 83.21± 1.10% for 400,200,100,50 and 25 µg/ml respectively within IC50 of 98.54 µg/ml. A little effect on viability of normal cells were observed after collagen treatment (72.33±2.41, 76.27±5.12, 83.17±1.31, 94.56±1.51 and 94.13±0.99 %) for 400,200,100,50 and 25 µg/ml respectively within IC50 of 122 µg/ml.

Keywords: Collagen; DPPH; Antioxidant; Anticancer

1 Introduction

Collagen is the quintessentially dominant protein in connective tissue, and it is found in a wide range of species in the tissues of all kinds of multicellular organisms; it workouts increased than a few functions, relying on its place (1). It can be extracted from extraordinarily a range animal species and it is in many instances derived from slaughter by-products. The critical sources of collagen are skin, tendons, cartilage, beef and bones. Some researchers have turned to methods of extracting collagen from refined animal sources such as fish and poultry (2).

There is developing exercising in the techniques used to extract collagen and its derivatives due to the developing tendency to use this protein in neighborhood of artificial marketers in a vary of industrial processes, and in addition to furnish an increased appreciation of the by-products of animal slaughter (3). Collagen is one of the most really helpful biomaterials due to the reality it has a massive fluctuate of industrial features (4). Therefore, the use of plant antioxidants is changing the use of these artificial antioxidant elements due to their safety, nutritional and therapeutic value.

In present day years, the antioxidants using as an ingredient and clever materials in people's weight loss system has significantly raised. A frequent ingredient that is considered an antioxidant is collagen in its hydrolyzed structure.

The plant peptides used as collagen hydrolysate has been dramatically exploited due to their accurate and extraordinarily biocompatibility, handy biodegradability, and inclined antigenicity (5). Some protein hydrolysates from casein, wheat gluten, lactalbumin, and soy have been studied for residence of antiaging and renewal fibroblast in monolayers. Celle culture (6). However, an entire lot an awful lot much less is recognized about the advantages of HC

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on the pores and skin and its habitat as an anti-aging agent, UV protector, the fibroblast and extracellular matrix (ECM) enhancer, moisturizer and nutrient.

Guided tissue regeneration (GTR) is a modality used to manage the early dynamics of periodontal surgical wound repair to promote periodontal regeneration (7). In addition, it has been used for bone defects and pre-implantation defects, as well as bone augmentation strategies prior to implant placement (8). The precept of GTR is to use the membrane as a mechanical barrier to protect the thrombus, create accommodation, and facilitate repopulation of the defect with regeneratively driven cells (9). Several factors have been used as barriers, in the unique polytetrafluoroethylene (PTFE), collagen, polyglycolic acid and copolymers (10).

Thus, many bioabsorbable barrier sellers have been used clinically in the GTR device (11). These boundaries are now created the usage of collagen synthesis the use of the cross-linking approach (8) to decrease the fast resorption and imbalance in the surgical area. However, the approach used to expand the cross-links between collagen molecules, increasing the stiffness and reducing the resorption of the collagen barrier, may further prolong its cytotoxicity.

2 Material and Methods

2.1 Extraction of collagen from buffalo

The collagens in this search have been extracted the usage of the techniques described in (12). with some modifications. All extraction techniques had been accomplished at four °C in continuous of stirring. To get rid of non-collagenous proteins from these samples, the meat was once as soon as blended as soon as with 0.1 N NaOH at a sample/alkali response ratio (w/v) of 1:10 for six hrs. The alkaline response modified in each and every two hrs. The protein-free pores, and skin have been washed on a lot of situations in a cold water till an intention or negligible wash water pH was once as quickly as acquired. The skin-releasing protein was once as soon as defatted by (10%) butyl alcohol at a solid to solvent ratio of 1:10 (w/v) for 18 hrs, and the solvent used to be as soon as modified every and every 6 hrs. The defatted meat must be consistently washed by cold water and then determined thru soaking in 0.5 M of acetic acid with a solid/solvent ratio of (w/v) 1:30 for 24 hrs. The mixture then filtered as soon as. The residue was re-extraction does now no longer meet the required stipulations for analysis. Both filtrates have been combined. Once, collagen is quicker or later precipitated through functionality of which consist of NaCl to a closing interest of 2.6 M in the presence of 0.05 M tris(hydroxymethyl)aminomethane, with pH 7.0. The pellet then used to be as soon as accrued as rapidly as attainable by the aid of the using of centrifugation at 4000 rpm for 15 minutes. The precipitate ensuing used to be dissolved as quickly as in 0.5 M acetic acid and dialyzed into 0.1 M acetic acid and distilled water, respectively, and then freeze-dried. The natural triple helix form and fibrillar form of collagen have been precisely preserved in the extraction techniques.

2.2 DPPH antioxidant activity of collagen

The antioxidant hobby of collagen and proper diagnosed (vitamin C) have been assessed on the groundwork of the radical scavenging have an effect on the constant DPPH free radical, and the approach of (13). used to be as quickly as followed. An aliquot of 0.1 ml of collagen or fashionable (200, 100, 50, 25 and 12.5 mg/ml) used to be as soon as as quickly as delivered to 3.9 ml of DPPH reply looking at the tube. After incubation at 37°C for 30 minutes, the opt.used to be decided at 517nm the utilization of spectrophotometer. The doable to scavenge DPPH radical used to be as soon as quickly as calculated through the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \right) \times 100$$

2.3 Cytotoxic Effect of collagen on MCF-7 breast cancer cell line using MTT assay

After cell line renovation in accordance to (14)., and reap ultimate cell hobby that used to be carried out through counting the cells the utilization of the hemocytometer and making use formulas:

$$\text{Total cells/mL: number of cell zes} \times \text{dilution question (or sample volume)} \times 104.$$

In this test, exceptional concentrations (12.5, 25, 50, 100, 200 and four hundred µg/ml) of collagen had been used for a attribute MTT assay the use of an equipped batch deal consisting of 1 ml of MTT response. × 10 bottles and 50 ml of solubilizing answer × two bottles. Briefly, tumor cells (1x10⁴–1x10⁶ cells/ml) have been grown in ninety-six flat appropriate microtiter plates in a remaining quantity of 200 µl of entire medium per terrific sterilized parafilm-coated microplate and incubated at 37 °C, 5 percentage CO₂ for 24 hours. After the medium used to be discarded, successive

concentrations of collagen (12.5, 25, 50, 100, 200, and four hundred $\mu\text{g/ml}$) have been brought to the wells. The plates had been incubated underneath temptation. 37°C , 5 percentage CO_2 for (24 h), then, 10 μl of MTT response was once as soon as as quickly as delivered to every appropriate and in addition incubated at 37°C , 5 percentage CO_2 for four h. The medium was once cautiously removed, and one hundred μl of solubilizing answer used to be brought to every properly for 5 min. finally, the usage of an ELISA reader to measure at a wavelength of 575 nm. Optical density documents used to be subjected to statistical evaluation to think about the interest of connections needed to bring down the cell 50% lifetime price for each cell line (15).

3 Results

3.1 Antioxidant activity of collagen by DPPH radical scavenging activity

Collagen was once as soon as large in DPPH radical scavenging workout routines at all concentrations examined (12.5, 25, 50, 100, 200 mg/ml). Concentrations of one hundred and 200 mg/ml collagen shared the approximate scavenging of radicals (54.44 ± 2.05 and $67.86 \pm 3.65\%$, respectively). Vitamin C moreover demonstrated variations between all concentrations in the vary from (22.90 ± 1.83 to 83.37 ± 1.85) for 12.5 and 200 mg/ml, respectively (Figure 1).

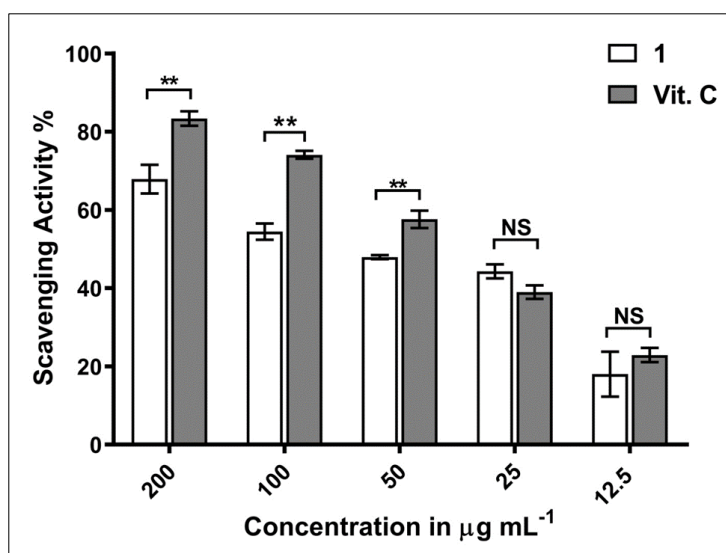


Figure 1 DPPH radical scavenging activity of collagen and vitamin C, [1: represented collagen]

3.2 Cytotoxic activity of collagen on MCF-7 cancer cell line

Table 1 Cytotoxicity effect of collagen on MCF-7 and WRI-68 cells after 24 hours incubation at 37°C

Concentrations $\mu\text{g/ml}$	MCF-7	WRL-68
	Viable cell counts (Mean \pm S.D.)	Viable cell counts (Mean \pm S.D.)
400	40.20 \pm 3.06	72.33 \pm 2.41
200	48.64 \pm 1.48	76.27 \pm 5.12
100	61.57 \pm 1.33	83.17 \pm 1.31
50	71.14 \pm 0.82	94.56 \pm 1.51
25	83.21 \pm 1.10	94.13 \pm 0.99

The 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) technique used for the beforehand completed to infer the cytotoxic impact of collagen on the breast most cancers cell line (MCF-7) to calculate cell viability and cost inhibition. The tumor hotline thru the use of one-of-a-kind collagen concentrations. The fraction of viability of handled cells was once calculated as soon as when evaluated the use of the traditional cell line WPL-68. Cytotoxic outcomes of inexperienced synthetic NPs in focus in the vary of 25 to 400 $\mu\text{g/ml}$ on MCF-7 cells (Table 1) led to a reduce in cell viability in of a dose-dependent. The cell's viability is declining due to the developing attention of collagen. The limit in

MCF-7 cell viability (%) was once as soon as referred to at four hundred $\mu\text{g/ml}$ ($40.20 \pm 3.06\%$), whilst the high-quality MCF-7 cell viability was once carried out at 25 $\mu\text{g/ml}$ ($83.21 \pm 1,10\%$). Collagen confirmed markedly the sturdiest cytotoxic exercising with an IC_{50} of 98.54 $\mu\text{g/ml}$. again, an IC_{50} of 122 $\mu\text{g/ml}$ was once as soon as got by means of publicity of collagen to each day cell line WRI-68.

4 Discussion

Collagen is enormous internal the man or woman physique and extracellular matrix neighborhood of molecules that holds at the same time the body's tissues. the protein in the human being physique and three quarters of the dry weight of pores and pores and skin consist of collagen. performs a key attribute in the natural enchancement of wound healing reducing contamination attracting cells that promote wound healing (16). The collagen antioxidant pastime has been influenced via the usage of the compositions, constructions and amino acid hydrophobicity, and collagen molecular weight (17). The three the dominant amino acids in these collagens showcase these non-polar amino acids, so collagen must commonly be hydrophobic. Antioxidant endeavor that seems to inhibit the formation of first-rate radical chains. Collagen is a protein that consists of three polypeptide chains collectively in a triple helical shape and has three discontinuous residues in the structure of Gly-X-Y. Glycine is the foremost amino acid fashioned in collagen, even although the X issue was once as suitable as the Y for hydroxyproline. (18). The shape and function of amino acids in the peptide chain are key factors in identifying the antioxidant exercise of collagen. Inhibition of chain reactions for all free radicals was once made with the beneficial aid of immoderate content material of proline and glycine in the tissue cloth in the amino acid sequence of collagen, which play the position of proton donors in relation to free radicals.. (19). An extra risk is the presence of -OH and -NH₂ companies in collagen peptides, which favorably bind to free radicals.. (20). Collagen amino acid for pores and pores and pores and skin parang-parang dominates with the aid of glycine, proline, glutamic amino acid, alanine and arginine. The complete hydrophobic amino acid (THAA) suggests a excessive degree of fats solubility and extends antioxidant activity. The C- or N-peptide termini in the hydrophobic amino acid chain can engage with lipid molecules and make contributions to free radical protonation. In addition, the relationship between molecular weight influencing antioxidants is shown, which are peptides that collect antioxidant fascination when many situations consist of 2–20 amino acids with molecular weights nicely under 3000 Da. (21).

5 Conclusion

Overall, the results of this study demonstrated that collagen extracted from buffalo meat exhibited a dose-dependent free-radicle scavenging activity with considerable cytotoxic capability against breast cancer cells.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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