Proteasome Inhibitory Potential of Commonly Consumed Dietary Ingredients

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Abstract- Consumption of fruits, vegetables and spices is associated with a reduced risk of cancer. Polyphenols, the predominant phytochemicals in dietary sources are known to have health beneficial effects. Flavonoids are the most potent polyphenols which have anti-allergic, antioxidant, anti-inflammatory, anti-viral and anti-cancer activities. Some flavonoids have recently, been reported to inhibit proteasome activity. The 26S proteasome, a multi-enzymatic complex present in eukaryotic cells, is a part of the ubiquitin proteasome pathway (UPP), the major cellular proteolytic pathway. Cancer cells have high proteasome activity, essential for their growth and survival. Inhibition of the proteolytic activity of the proteasome leads to cancer cell death. We screened a few dietary constituents belonging to different food groups rich in phenolic content for the total phenolic and flavonoid content and proteasome inhibitory activity using standard protocols. All the dietary constituents tested could inhibit the chymotrypsin-like activity of the purified 20S proteasome enzyme in vitro, albeit at different levels. Among the ingredients tested, cinnamon and curry leaves had the highest inhibitory potential. These food ingredients need to be further explored for their anticancer potential.

Index terms- polyphenol, flavonoid, proteasome inhibitor, dietary ingredient

I. INTRODUCTION

Polyphenols, a diverse class of phytochemicals widely distributed in nature arise through one of the two metabolic pathways: the ‘shikimate pathway’ or the ‘polyketide pathway’ (acetate pathway). They are defined chemically as substances having more than one phenol unit. Flavonoids are the largest and most important group of polyphenols that are ubiquitous in plants [1]. Flavonoids are water soluble compounds containing 15 carbon atoms; represent a wide class of phenolic phytochemicals which constitute an important component of the human diet. They are found in fruits, vegetables, spices, and beverages, providing them with much of their flavour and colour [2]. In addition to the endogenous antioxidant systems (catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase), exogenous antioxidants play a vital role in protecting against oxidative stress. These exogenous antioxidants include vitamins, carotenoids and polyphenols [3]. Eukaryotic cells perform the vast majority of their regulated proteolysis through the ubiquitin proteasome pathway (UPP). The 26S proteasome, often called the “proteasome”, is a multi-catalytic enzyme complex expressed both in the nucleus and cytoplasm of all eukaryotic cells. This is the major intracellular, extra-lysosomal, proteolytic system involved in proteolysis. Its highly selective regulated actions ensure rapid degradation of target proteins [4]. The proteasome is a 2.4MDa complex that consists of the two large subunits, the 20S catalytic core particle and the 19S regulatory particle. The 20S subunit possesses at least three distinct activities, which are associated with the three different β subunits respectively: chymotrypsin-like activity (β5), trypsin-like activity (β2) and the caspase-like activity (β1), the chymotrypsin-like activity being the rate-limiting step of protein degradation. The 19S particle regulates the function of the 20S core particle and controls the access of substrates into the proteolytic core [5]. The UPP is critical to a number of cellular processes, including the progression of the cell cycle, oncogenesis, apoptosis, selective elimination of misfolded proteins, and antigen processing [6]. In recent years synthetic polyphenols/flavonoids have been reported to possess proteasome-inhibitory activity and also act as anticancer agents [7], [8], and [9]. In the present work, we screened dietary constituents from different food groups that are reported to be rich in phenolic content, namely green leafy vegetables, fresh fruits, dry fruits and spices for total phenolic and flavonoid content and proteasome-inhibitory activity. We observed that all the dietary constituents tested had high total phenolic content and varying amounts of total flavonoids. The inhibition of the chymotrypsin-like (Ch-L) activity of the 20S proteasome by the dietary ingredient which is known to be critical for protein degradation was measured. It was observed that all the constituents from the different food groups could inhibit the Ch-L activity of the purified 20S proteasome, which is critical for protein degradation albeit at different levels.

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II. MATERIALS AND METHODS

A. Chemicals and Reagents

20S proteasome purified from rabbit was purchased from Boston Biochem, USA. The fluorogenic proteasomal peptide substrate, Suc-Leu-Leu-Val-Tyr-AMC (Suc-LLVY-AMC for chymotrypsin-like activity) was procured from ENZO Life sciences, USA. Gallic Acid and Quercetin used as standards, N-(2-Hydroxyethyl) piperazine-N′(2-ethanesulfonic acid) (HEPES) sodium dodecyl sulfate (SDS) and aluminium chloride were purchased from Sigma Aldrich, USA. All other reagents were of analytical grade and were procured from Qualigens Fine Chemicals Mumbai, India.

B. Extraction of total polyphenol from dietary ingredient:

3-5g of dietary ingredient was weighed and ground to a fine powder in a dry grinder. To the ground powder or pulp (in case of wet ingredient) was added 80% methanol (extraction solvent), vortexed for 2 minutes and incubated at room temperature for 1h. This was centrifuged at 8,000 rpm for 15 minutes at 10ºC. The supernatant was collected and the residue was re-extracted with 80% methanol as mentioned above. The two supernatants were pooled to get a 10% extract. The pooled supernatant was filtered using a 0.45µm filter (Millipore, USA) and the resultant Methanol: Water extract was stored at -20ºC till further use. Three independent extractions were done for all the dietary constituents.

C. Total Phenolic Content of the dietary ingredients (TPC):

The total phenolic content of the food extracts was determined by the Folin–Ciocalteau’s method [10] using Gallic Acid as a standard. Different concentrations of Gallic Acid standards (20-100µg/µl) or food extract samples were taken in glass test tubes and the volume made upto 150µl with distilled water. 750µl of 10% Folin’s reagent was added and kept at room temperature for 5 minutes, followed by addition of 750µl of 6% NaHCO3 and vortexed for 5 minutes. The tubes were then incubated for 90 minutes at room temperature and the absorbance measured at 725nm in a Hitachi double beam spectrophotometer. The total polyphenol content in the extract was expressed as mg of Gallic Acid Equivalents (GAE) per 100g fresh weight. The assay was performed in three independent samples (in triplicates) and the data is expressed as mean +/- SEM.

D. Total Flavonoid Content of dietary ingredients (TFC):

The total flavonoid content of the food extracts was estimated by the Aluminium Chloride method [11] using Quercetin as a standard. Different concentrations of quercetin standards (10µg-100µg) or the food extract were taken and the volume was made up to 1ml with 80% methanol. 500µ of 10% AlCl3 solution was added to the standards and the samples and vortexed. Then 500µl of 1M CH3COOK solution was added and again vortexed. 2ml of double distilled water was added to the standards and samples and vortexed. The tubes were incubated at room temperature for 30 minutes. The absorbance was read at 415nm in a spectrophotometer. The total flavonoid content in the extract was expressed as mg of Quercetin Equivalents (QE)/100g of fresh weight. The assay was done in three independent samples (in triplicates) and the data is expressed as mean +/- SEM.

E. Inhibition of the Chymotrypsin-like activity of the 20S proteasome by the dietary ingredient:

The inhibition of chymotrypsin-like activity by the food extracts was measured in vitro using the purified rabbit 20S proteasome [12]. In brief, 100ng of purified 20S proteasome was incubated in 200µl of assay buffer (50 mM Tris-HCl, pH 8.0 containing 0.035% SDS) with or without the food extract equivalent to 5µg GAE (in triplicate) and 40µM of the substrate Suc-LLVY-AMC (specific for the chymotrypsin-like activity) and incubated for 2h at 37°C. The free 7-amino-4-methylcoumarin (AMC) liberated by substrate hydrolysis was measured fluorimetrically using a multi-mode reader [Spectra Max M5] using an excitation filter (380nm) and emission filter (460nm). The assay was done in three independent samples (in triplicates) and the data is expressed as mean +/- SEM. The data is expressed as a percentage of the control, which was considered to be 100%. The percentage inhibition was calculated as follows:

Percentage Inhibition (%): 100 – X where X= Fluorescence (RFU) in the presence of inhibitor X 100/ Fluorescence (RFU) in the absence of inhibitor.

F. Statistical Analysis:

Three independent samples were analyzed for all the dietary ingredients. All data are expressed as Mean +/- SEM. Descriptive statistical analysis was done for the three variables (TPC, TFC and Inhibition of Ch-L activity). To study the relationship of the three variables Spearman’s Rank Correlation Coefficient was used. Level of significance (p) was calculated as < = 0.05. SPSS version 19 (IBM Corp, Somers, NY, USA) was used for statistical analysis.

III. RESULTS

Dietary constituents from different food groups that have high phenolic content (>250mg/100g fresh wt) were used for screening. The different food groups and the dietary ingredients tested are shown in table 1.
Among all the ingredients, clove had the highest total polyphenol content of 27.6g of GAE/100g of clove and mint had the lowest polyphenol content of 0.24g of GAE/100g of mint. Curry leaves among green leafy vegetables, apricot among dry fruits and clove from the spices group had the highest polyphenolic content. Among all the food groups, spices were found to have the highest polyphenolic content with the exception of pepper. The data for the total polyphenolic content is depicted in table 2.

Among the dietary ingredients tested, clove had the highest total flavonoid content. This was followed by amaranth, curry leaves and apricot which also showed high flavonoid content. In general, there was a weak positive correlation ($r^2 = 0.411$) between the total polyphenol content and total flavonoid content among the dietary ingredients tested. However, clove, apricot and curry leaves that had a high TPC also had high TFC. It was observed that the highest flavonoid content was in clove (653.3mg QE/100g of clove) and the lowest was observed in guava (11.6mg QE/100g of guava). The flavonoid content of pepper could not be estimated due to turbidity in the sample. The total flavonoid content of the dietary ingredients is depicted in figure 1.

The inhibition of the Ch-L activity of the proteasome was assessed in vitro using a purified rabbit 20S proteasome enzyme. It was observed that cinnamon had the highest inhibitory activity followed by curry leaves and guava. Although apricot had high polyphenol content, the % inhibition was lower.
On the contrary, though the TPC of guava was not high the inhibition of Ch-L activity of the 20S proteasome was higher. Among the dietary ingredients tested, amaranth showed the least inhibitory activity. The inhibitory activity in pepper could not be estimated due to turbidity in the sample. The data is shown in figure 2.

Figure 1. Total Flavonoid Content of Dietary Ingredients.

Figure 2. Inhibition of the Chymotrypsin-like Activity of the 20S proteasome by the dietary Ingredients

IV. DISCUSSION

Foods and beverages rich in phenolic content, specially the flavonoids, are often associated with decreased risk of developing several diseases. Among polyphenols, flavonoids have many health-promoting effects due to their anti-allergic, anti-oxidant, anti-inflammatory, anti-viral and anti-tumour activities. In recent years some synthetic polyphenols such as Apigenin, Quercetin and Epigallocatechin-3-gallate (EGCG) inhibit the chymotrypsin-like activity of the 20S proteasome [7], [8]. *In vitro* and *in vivo* studies have shown that these synthetic polyphenols induce apoptosis in human cancer cells [9].

Accumulating evidence indicates that the ubiquitin proteasome pathway (UPP) plays an essential role in cancer. In recent years human neoplastic cells have been shown to possess very high proteasome activity, which is required for their growth and survival [13]. Keeping in view the cellular processes that the UPP controls, proteasome inhibition deregulates signalling cascades ultimately resulting in apoptosis. Since transformed cells have faster proliferative rates and defective cell cycle checkpoints, they are more vulnerable to pro-apoptotic stimuli than normal/non-transformed cells [14]. Importantly, inhibitors of the 20S proteasome have been shown to induce apoptosis and cell cycle arrest in neoplastic cells only. Therefore, the proteasome has now emerged as an attractive molecular target for cancer therapy. Numerous drugs that inhibit the proteasome have been described, many of which interfere directly with the proteolytic activity of the 20S core particle. They include the synthetic peptide aldehydes such as MG-132, non-peptide inhibitors such as lactacystin and epoxomicin, and the peptide boronic acids [12]. Many of these compounds have broad specificity, poor metabolic stability, and bind irreversibly to the proteasome.

The search for new anticancer drugs from natural sources is one of the most important approaches for cancer prevention and therapy. In this study we screened few dietary ingredients from different food groups for their total polyphenolic and flavonoid content and also their proteasome inhibitory potential. It was observed that all the ingredients tested could inhibit the chymotrypsin-like activity of the 20S proteasome. There was a weak positive correlation between the TPC and TFC suggesting that some food ingredients that had high TPC also had high TFC. In this study we screened few dietary ingredients from different food groups for their total polyphenolic and flavonoid content and also their proteasome inhibitory potential. It was observed that all the ingredients tested could inhibit the chymotrypsin-like activity of the 20S proteasome. There was a weak positive correlation between the TPC and TFC suggesting that some food ingredients that had high TPC also had high TFC. However, there was no correlation between the TPC/TFC and the inhibition of Ch-L activity of the 20S proteasome. This suggests that the dietary ingredients may possess certain other polyphenolic compounds such as tannins and alkaloids apart from flavonoids, which could also contribute to their proteasome-inhibitory potential. Recently, tannins and alkaloids have also been reported to have proteasome-inhibitory activity [15, 16]. Therefore, proteasome inhibitors from dietary sources with minimal or no toxicity can be potential anticancer agents. Hence, active component(s) need to be isolated and characterized from the different dietary ingredients and tested for anticancer activity.

V. CONCLUSION

The present study demonstrates that dietary constituents that are rich in phenolic content can inhibit the chymotrypsin-like activity of the 20S proteasome. Our work emphasizes the need to explore and incorporate polyphenolic and flavonoid rich foods in cancer therapy. Therefore, these dietary constituents can be tested for their potential as anticancer agents in human cancer cell lines and animal models.
REFERENCES


