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RESEARCH ARTICLE

Evaluation of Antioxidant Efficiency of Nanosized Quercetin: An *In Vitro* Study

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Abstract

Quercetin is a bioactive compound that is widely used in botanical medicine due to its powerful antioxidant activity. This work aims at encapsulated of quercetin into PLGA nanoparticles by using the nano participation technique. Following by the valuation of their functionality as antioxidant activity. Quercetin and quercetin nanoparticles by using straight forward in vitro free radical scavenging systems including DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical-scavenging assay and hydrogen peroxide radical scavenging. Quercetin nanoparticles showed strong scavenging effects against DPPH, hydrogen peroxide and this effect was more potent than pure quercetin. The scavenging activity of quercetin nanoparticles was concentrations dependent in the range of 4-12 µg mL^{-1} showing maximum activity of 90% at 12 µg- mL^{-1}in similar way to Vit.C which was used as positive control. In conclusion, this work displayed that quercetin exhibited antioxidant activity and could be considered as a source of antioxidants from nature.

Keywords: *Quercetin nanoparticles, Antioxidants, DPPH, DNA damage.*

Introduction

Recently, there is an increasing in antioxidants interesting. The main reason for this interesting due to the protection organelles of the cells, specially membranes and the pathways metabolic against the oxygen free radicals and their reacting derivatives (ROS). They are participating in formulation of chronic inflammatory states and other diseases associate with oxidative stressed, such as atherosclerosis, cancer, hypertension, neurodegenerative, and cardiovascular diseases [1].Anti-oxidant components from dietary's source are receive enormous awareness.

Amongst them are flavonoids are one of the most important group of natural substance [2]. They belong to a classes of secondary metabolites, with a variable polyphenolic structure, are widely present in vegetables, leaves, flowers, fruits and certain beverage. Flavonoids involved various appropriate biochemical and anti-oxidant effective by exhibiting biological activities with a various diseases such as Alzheimer's disease, cancer [3, 4]. They are well known for their beneficial effects on health. Flavonoids necessary components in a diversity of pharmaceutical, medicinal, and nutraceutical, applications. Related to their properties as, anti-inflammatory, anti-oxidative, anti-carcinogenic and anti-mutagenic properties. Also with their ability to modulated key cellular enzyme functions. Flavonoid showed higher activity as inhibitors for many enzymes such as, lipoxygenase, cyclo-oxygenase (COX) and phosphoinositide -3-kinase most commonly-consumed flavonoids are quercetin [5,6].

Quercetin (3, 3', 4, 5, 7-pentahydroxy-flavone) which belong to polyphenolic flavonoids compound [7].It is abundantly present in onions, berries, apples, red grapes, broccoli, and cherries, as well as tea [8]. Quercetin is a yellow color, crystalline solid with a bitter taste, and poorly soluble in hot water, quite soluble in alcohol, lipids, aqueous alkaline

solutions and soluble in glacial acetic acid [9]. Modern studies have shown that quercetin prevents numerous diseases such as cardiovascular, osteoporosis (porous bone) and some forms of cancer [10]. In addition, Quercetin is consider to be a strong antioxidant due to its ability to scavenging free radicals and chelating transition metal ions.

These properties of quercetin allow it to inhibition lipid peroxidation [11]. Lipid peroxidation is the process by which polyunsaturated fatty acids are converted to free radicals by the abstraction of hydrogen ions. [12] Lipid peroxidation can created harmful effects through the body. Such as, neurodegenerative and cardiovascular diseases. And also capacity by regulation level of glutathione (GSH) [13].

In spite of this wide beneficial properties for quercetin, but it`s limited to use because of bioavailability is very poor, it's has very low solubility in water, intestinal permeability, instability and easy degradable, One way to overcome these problems is to encapsulation these antioxidant into nanoparticles [14, 15]. Biodegradable polymers have played an important role for the encapsulated of many antioxidants because of its biodegradability, non- toxicity, hydrophobicity, and sustained-release [16].

Quercetin has been successfully encapsulation into Poly lactic-co-glycolic acid (PLGA) is one of the extensively researched synthetic biodegradable polymers due to it's favorable properties. PLGA is approved by the US FDA (food and drug administration) for several therapeutic applications because of it's biocompatibility, protection from chemical instability, non-immunogenic polymer, possibility to improvement surface properties to make more interaction with biological materials and targeted drug delivery system [17,18].

The objective of the present study was to develop a quercetin loaded polymeric (PLGA) nanoparticles and estimate the antioxidant activity by accomplished different in vitro assays.

Material and Methods

Acetone (AFCO, Jordan), PLGA poly (D, L-lactide-co-glycolide), quercetin Poloxamer 407, ascorbic acid (Vitamin C) and 1-diphenyl-2-picrylhydrazyl (DPPH), were purchased from Sigma (St. Louis, USA), Standard fish DNA (BDH, England).

Fabrication of Quercetin- loaded PLGA /Poloxamer 407 Nanoparticles

Quercetin loaded PLGA were prepared using nanoprecipitation method as previously reported by Sulaiman et al. with some modifications [19]. In Brief, 20mg of quercetin and 50 mg of PLGA were dissolved in 1.25mL of acetone and mixture together. Then, the solution kept immediately on mechanical stirrer at 2000 rpm for 30min at 25°C. Followed by dissolved 50mg of Poloxamer (407) was used as a surfactant in 5mL of (deionized water). The organic phase containing the PLGA and active ingredient was then added to the aqueous phase drop by drop by using burette directly. Then the solution transferred to 20mL (deionized water) to simplify diffusion and kept under magnetic stirrer overnight at 25°C. The homogenous solution was centrifuged at 13,000 rpm for 20min to separated free unbound compounds, stabilizer in solution.

Antioxidant Activities Evaluation

DPPH (1, 1-diphenyl-2picrylhydrazyl) radical scavenging activity of Quercetin-PLGA nanoparticles was valuated as followed according to Ali and co-workers with some modification [20]. Typically, absolute ethanol 500µL was add to different tubes and mixed with 0.5mL different concentrations of quercetin and nanoquercetin (4, 6, 8, 10 and 12 µg mL^{-1}) and then 500µL of (DPPH) (60µM) was added into all tubes and brooded about 30 min at room temperature.

The negative control was DPPH with ethanol only and the positive control was 5 µg mL^{-1} of vitamin C. After that the absorbance of all samples was tested at 517 nm utilizing UV-VIS spectrophotometry (Metertech, Germany). The percent of DPPH scavenging efficacy was measured utilizing the next equation:

Anti-oxidant capacity (%) =Ac-As\Ac*100

Ac represents the peak intensity of DPPH. While, as is the peak of quercetin or quercetin nanoparticles sample solvent.

Determination of DNA Damage Spectrophotometrically

Quantification of antioxidant capacity was determined spectrophotometrically by using DNA damage assay. In this assay, the absorbance ratio of DNA sample about (A260/A280) which treated with a different concentrations (5, 10, 15, 20, 25 µg mL^{-1}), equal volumes of quercetin , quercetin -PLGA nanoparticles and standard DNA solution were mixture with subsistence of 1×10^{-5} M of (H_2O_2) hydrogen peroxide solution followed incubated about 10 min at 37°C temperature , after this period the effect was determined by evaluated the wavelength of the solution at 260 nm utilizing Ultraviolet-Visible spectrophotometer and in the current study 5mg mL^{-1} of Vitamin C Ascorbic acid was used as positive control [21].

Statistical Analysis

Data are presented was evaluated by using SPSS statistical program (Version/18.0; SPSS Inc., Chicago, IL). (ANOVA) was used for Analysis of variance to determine whether there are any statistically considerable differences between the means experiments. A p -values of < 0.05 was considered to be statistically significant.

Results and Discussion

Preparation of quercetin and quercetin-PLGA nanoparticles, the preparation based on nanoprecipitation technique. In these techniques, an organic solution of the PLGA polymer is emulsify in an aqueous solution with a surfactant. Then removing the organic solvent by stirring and this process permits formation of nanoparticles .as shown in Figures 1 and 2, respectively.

The difference in properties between the native quercetin and nanoquercetin wherease, the powder of nanoquercetin was a very fine dispersal and sprightly when compared with native quercetin as present in Fig.1 (B).And when nanoquercetin were re-suspended in water, it has more solubility, contrasting a pure quercetin which was completely unsolvable in water with undissolved particles clearly noticeable in the solution. While After 4 hr at 25°C temperature the nanoquercetin was remaining soluble in water, but a pure quercetin was totally precipitated (Fig. 2).

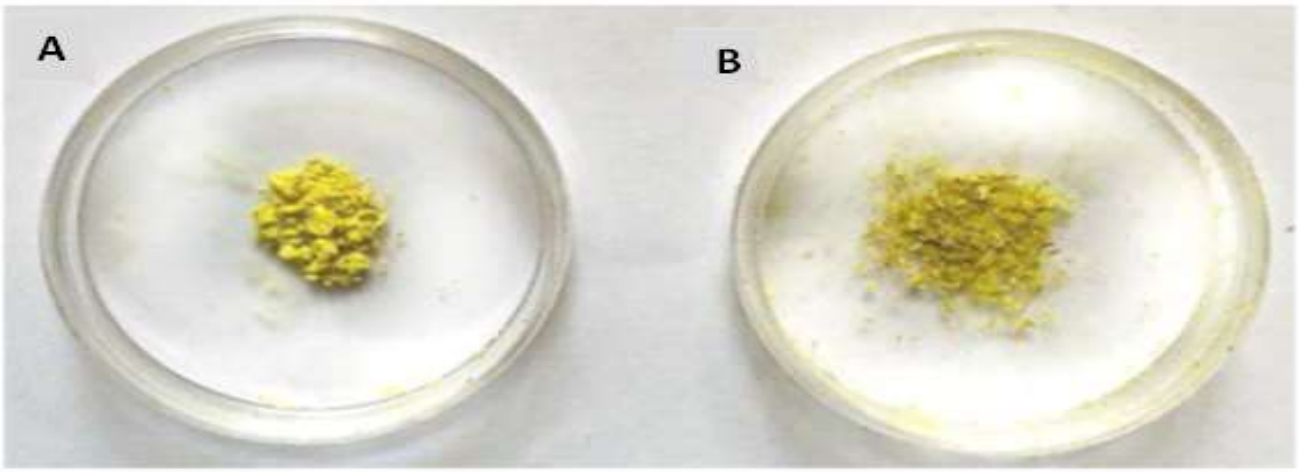

Fig.1: Image of (A) original quercetin and (B) modify quercetin nanoparticles powder

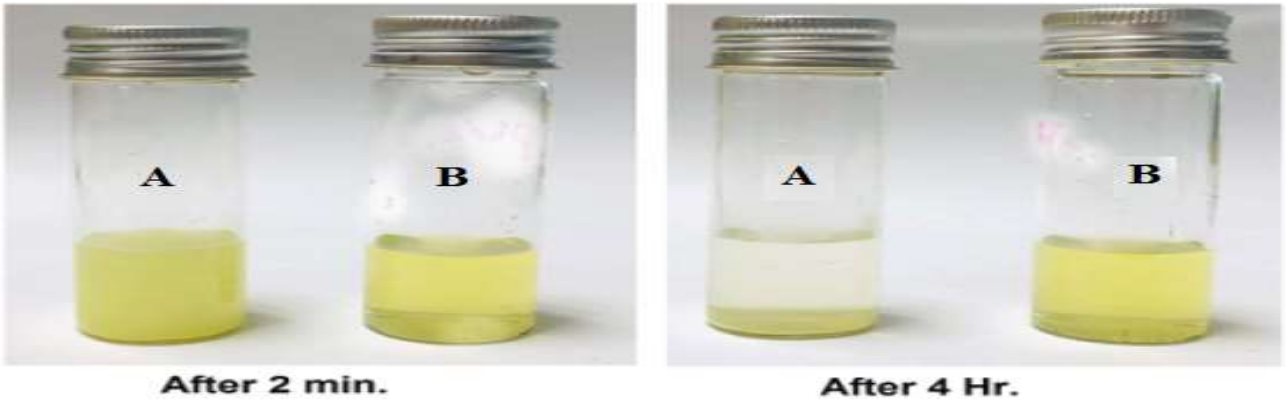

Fig.2: Image of solubility (A) original quercetin and (B) modifiy in water after 2 min and 4 h of preparation, respectively

Antioxidant Capacity of DPPH

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging process is a based on electron transfer that produces a deep purple colour in ethanol solution [21]. These free radical, stable at 25°C temperature is decrease upon reaction with an antioxidant molecule as hydrogen donor, to becomes colorless to pale yellow. And decrease in absorbance depends linearly on antioxidant concentration.

In Figure (3), showing the antioxidant activity of quercetin and quercetin loaded PLGA nanoparticles were observed used five various concentrations. The results show a higher scavenging ability of altered nanoquercetin compare with pure quercetin, and depending on the observed inhibition of concentration. The results illustrate that pure quercetin significantly decrease the level of the DPPH free radical dependent in a concentration manner at 12 μgmL^{-1} which was better than the other concentrations reaches 79.6% antioxidant activity while, 95.09% inhibition of positive control compared with nanoquercetin efficiency, that has higher activity of reduced the DPPH radical scavenging compare with pure quercetin which reaches 91.3% antioxidant activities.

The results indicated that quercetin entrapped in PLGA nanoparticles is more efficient in inhibition DPPH compare with pure quercetin which could be possibly explain by improved dissolvability.

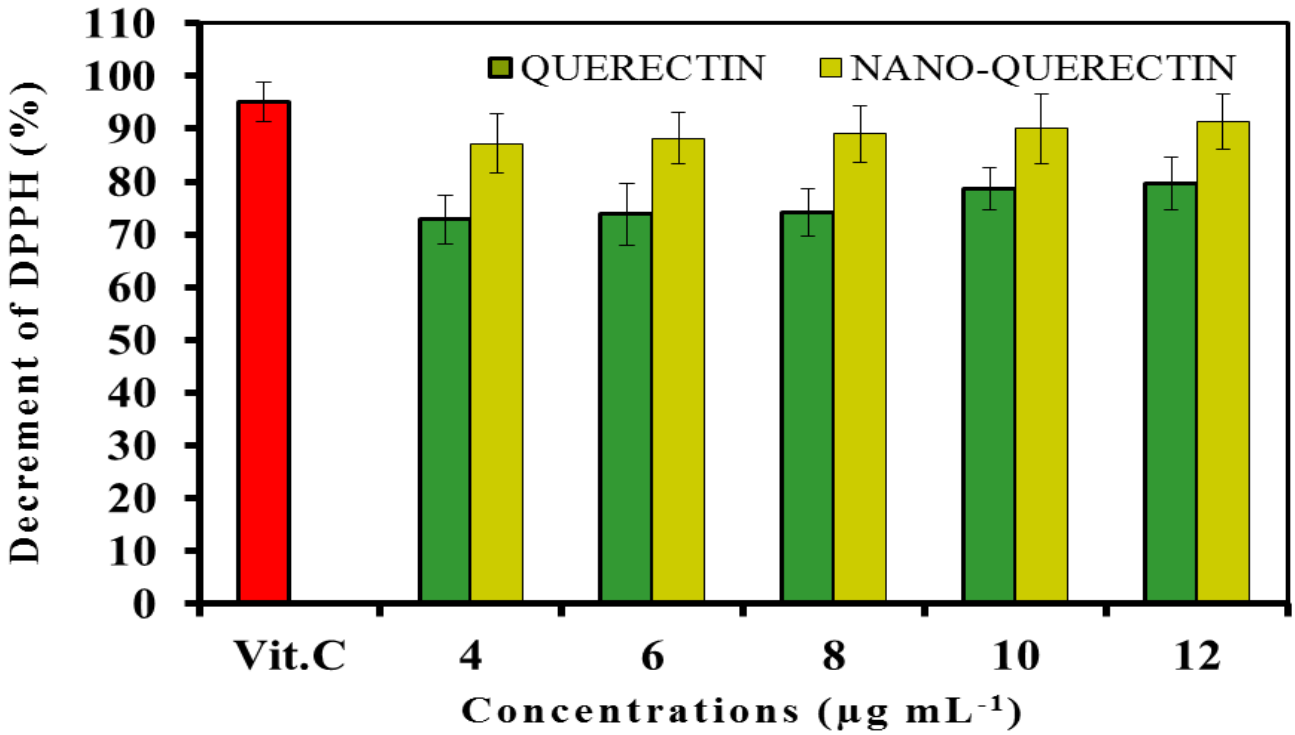

Fig. 3: DPPH free radical scavenging assay of quercetin and nanoquercetin

DNA Damage Assay

DNA damages stimulated by hydrogen peroxide (H_2O_2) are one of the appropriate model for measurement of protective capacity of pure quercetin and nanoquercetin.H_2O_2 is a non-radical compound but highly reactive, and is produced in high concentration in the living cells [23]. H_2O_2 is product of $O_2^{\cdot-}$ dismutation and other reactions mediated by different enzymes and participates in oxidation reaction through a non-radical pathway [24, 25]. Furthermore, the interaction of the superoxide ($O_2^{\cdot-}$) radical and H_2O_2 results in the formation of highly reactive hydroxyl radicals, which can be a source of more harmful species, such as hydroxyl radicals (OH) which are responsible for attacks guanine base and cause DNA damage. As shown in this figure [4] Adding pure quercetin or modified nanoquercetin in five concentration ($5\mu gmL^{-1}$ to $25\mu gmL^{-1}$) caused reduction in the H2O2 activity-induced DNA damage, and nanoquercetin was more effective in this regard; they show lesser absorbency when the concentration increases. And these related to the antioxidant property of modified nanoquercetin due to existence natural phenolic compounds consider as strong antioxidants.

So, it seems nanoquercetin is effective as therapy for free radicals associated diseases such as cancer, without harming normal cells.

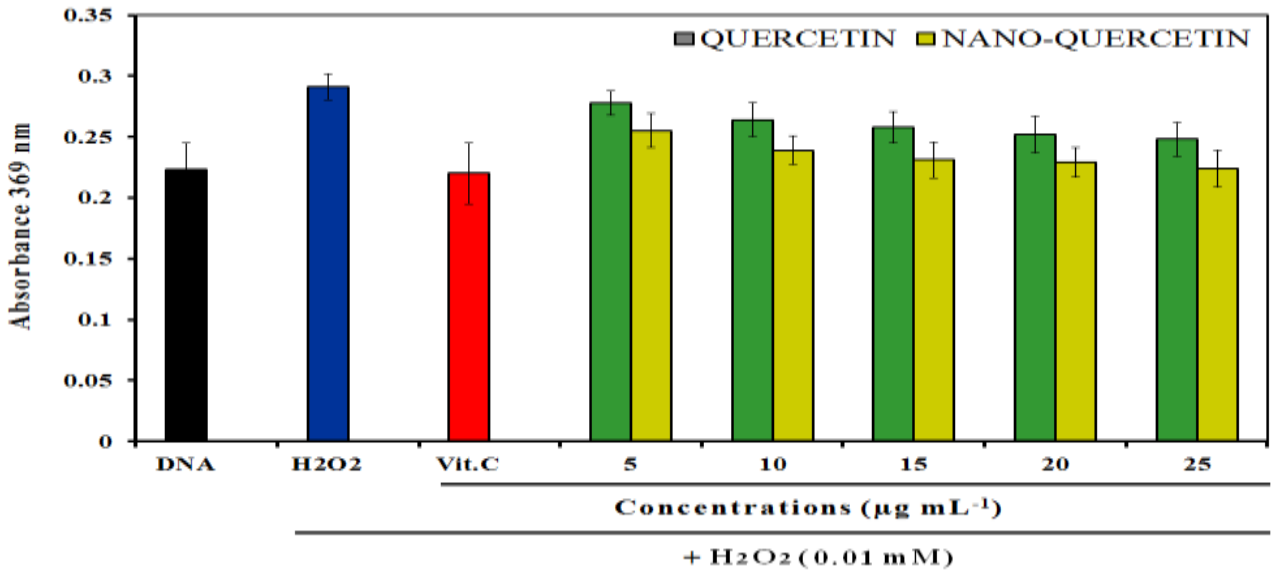

Fig. 4: Image showing structure of modify standard DNA sample, native quercetin and modified nanoquercetin loaded PLGA\ Polixamar 407

Conclusion

The presented results indicate quercetin-loaded PLGA-Poloxamer was successfully prepared by the nano participation method. Additionally, increase of antioxidant activities of nanoquercetin comparison with pure quercetin which is attributed to better dissolution property. Which making effectiveness by prevention diseases arising from oxidative stress. Furthermore make them attractive and favorable in the food and pharmaceutical industry.

Conflicts of Interest

All the authors declare that they have no financial or nonfinancial competing interests.

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