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Evaluation Of Antioxidant And Anti Inflammatory Activity Of Grape Seed Oil Infused With Silver Nanoparticles An In Vitro Study

Research Article

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Abstract

Aim: The aim of the current study is to assess the anti oxidant and anti inflammatory activity of grape seed oil gel which is infused with silver nanoparticle.
Materials And Methods: Materials and Methods: Grape seed oil gel infused with silver nanoparticle and the prepared gel was subjected to anti-inflammatory activity test with diclofenac sodium as standard preparation. Antioxidant properties of grape seed oil infused with silver nanoparticle was also tested with Butylatedhydroxytoulune BHT as control.
Results: From the results obtained it was seen that GSO gel showed and retained both anti oxidant and anti inflammatory-activity.It was observed that the anti oxidant activity of GSO gel was lesser than that of standard at all concentration but the differences are not statistically significant (p value 0.400)whereas in the experiment to test the anti inflammatory activity of GSO gel it as seen that the percentage of inhibition at all concentration except for the highest concentration was higher than that of the standard which is statistically significant (p value 0.045).
Conclusion: Grape seed extract is subjected to various changes during the synthesis of gel it is essential to check whether the properties are retained .From the results obtained it is seen that the anti oxidant and anti inflammatory activity increases as the concentration increases.

Introduction

Periodontitis is a chronic inflammatory disease caused mainly and primarily by bacteria in dental plaque. It affects the supporting structures of the teeth. Specific periodontal pathogens such as the gram-negative anaerobic bacteria present within the subgingival plaque are associated with the progression of the disease.

Although bacteria are the major etiological agents, the host immune response to these bacteria is also equally important, thus periodontitis is a multifactorial disease, controlled by the underlying immune and inflammatory responses of the host against the pathogens[18].

Free radicals & Reactive Oxygen Species (ROS) production is an essential component of the host response for immune system, formation of prostaglandin, development of anti-bacterial[29,24]. Major producers of reactive oxygen species are mitochondrial cytochrome P-450 reactions, peroxisomal fatty acid metabolism and NADPH oxidase activity[8,26].

An imbalance between the reactive oxygen species production and antioxidant mechanism leads to oxidative stress. Oxidative stress has been associated with both onset of periodontal destruction and systemic inflammation[7,3].

Grape is one of the most commercially important plant species due to its various uses like production of wine, grape juice and other food products[15,28]. Grape products are characterized by

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their metabolic compositions[34,37].

The major components of grape are phenols, and the phenols are mainly distributed in the skin, stem, leaf and seed of grape.In grapes, flavonoids are primarily located in the epidermal layer of berry skin and the seeds.

Flavonoids are the main groups of soluble phenolics in grapes as well as major contributors of the biological activities in products derived from grapes[4,39]. Flavonoids in grapes are the most feasible phytochemicals, responsible for this phenomenon. Flavonoids have cardioprotective, antioxidant, anti-inflammatory, anticancer and antimicrobial properties.

Antioxidant Action

ROS account for a wide range of aggressive free radicals produced by various metabolic pathways in living cells[21,31]. ROS are important regulatory agents in the complex signaling network of cells. They play a major role in promoting cell growth and differentiation, adaptation to metabolic and physiological stresses, immune response as well as protection from pathogen invasion. However, several factors may cause an over-accumulation of ROS by interrupting regular cellular processes and thus exposing tissues to conditions of oxidative stress[11]. When cells are exposed to oxidative stress they easily undergo oxidative damage that leads to a cascade of degenerative processes.

It is generally assumed that therapeutic treatment with antioxidants is the most effective way to control oxidative stress and to avoid occurrence of oxidative damage. Flavonoids are a large group of low molecular weight compounds with high antioxidant properties. Their specific chemical structure allows them to reduce oxidative stress through numerous mechanisms. because of their metabolic conversion in the human body, flavonoids generate large amounts of simple phenolic acids, which have significant effects in scavenging free radicals and improving the action of other antioxidants[38,19]. Flavonoids represent a large family of metabolites present in plants. The diversity in their chemical structures contributes to their broad range of physiological and biological activities. The most common flavonoids found in grapes are anthocyanins, flavonolscatechinepicatechin, dihydroflavanols and proanthocyanidins.

Anti-Inflammatory Action

Inflammation is a protective response of tissues against cell injury, irritation, pathogen invasions, as well as mechanism for eliminating damaged and necrotic cells. Several environmental stress factors may cause inflammation[40,16]. Under normal physiological conditions, anti-inflammatory cytokines act as immune regulators to control the inflammatory reactions. Deregulation of precise control mechanisms of inflammation leads to chronic inflammation and promotion of chronic disease[12,10]. Grape polyphenols have been shown to decrease chronic inflammation either by modulation of inflammatory pathways or by reducing ROS levels. As natural compounds, grape flavonoids and proanthocyanidins can target multiple pathways to overcome chronic inflammation, and thus are more effective compared to synthetic mono-targeted anti-inflammatory drugs.

It has also been demonstrated that proanthocyanidins in grape seeds have high anti-inflammatory action, because they scavenge free radicals, prevent lipid peroxidation and inhibit formation of pro-inflammatory radicals .Proanthocyanidins extracted from the grape seeds have also been found to have an immune-modulatory role in inflammatory conditions that exert an overproduction of nitric oxide and prostaglandin E2[32,6].

Herbal Gel Formulations

A gel is a soft, semi solid material which is a transitional state of matter containing both liquid and semi solids or semi-liquids[33]. Gels combine the cohesive properties of solids and the diffusive characteristics of fluids[5,13]. Many local drug delivery systems such as films, fibers, ointments or gels prepared using biodegradable or non degradable polymers have been tested for periodontal therapy. Among these carriers, gel formulations have received considerable attention in treating periodontitis[23,2,14]. Gel-based preparations are categorised into two main groups based on the polarity of the external liquid phase. hydrogels and oleo gels[20].

Gels are formed by the three-dimensional network of either natural or synthetic gelling agents to immobilise the aqueous phase. These formulations ensure better patient compliance because of their specific properties such as easy removal after application, cooling effect, a greaseless texture and good spreadability. Hydrogels exhibit high biocompatibility and mucoadhesive properties, as they can adhere to the mucosa in the dental pocket and reduce irritation at the site of application.

The GSO gel was prepared with carbopol , water and grape seed oil infused with silver nanoparticles whose characteristics and synthesis are mentioned in our previous study. The aim of the study is to assess the antioxidant and anti-inflammatory activity of grape seed oil gel which is infused with silver nanoparticles, since Grape seed extract is subjected to various changes during the synthesis of gel it is essential to check whether the properties are retained.

Materials And Methods

The in vitro study was conducted in the department of nano pharmacy and department of periodontics Saveetha dental college and hospitals, Chennai, India. Antioxidant and anti inflammatory activity for GSO gel was performed, The GSO gel infused with silver nanoparticle was prepared based on our previous study.

In brief the synthesis and development of GSO gel is as follows

Synthesis of silver nitrate nanoparticle infused with grape seed oil

In a flask to 9 ml of distilled water 1ml of grape seed oil was added and was heated at 90 degree centigrade until mild colour change was noted .To 90 ml of distilled water Silver nitrate was added (1 millimol) to this mixture the grape seed solution was added ant the conical flask was placed over a magnetic stirrer . The colour change in the solution was noted and the centrifugation process was done for 48 hours every 6 the solution was subjected to U-V spectrometric analysis and the amount of absorbance was analysed once the optimum value of 650 nanometers was achieved the solution was centrifuged at 8000 rpm the supernatant was dis-

carded ant the concentrate was used in the synthesis of gel. The gel preparation:Carbopol was taken in a beaker and dispersed in 50 ml of distilled water the beaker is kept aside for half an hour and then stirred at 1200 rpm for 15 minutes in another beaker 5 ml of propylene glycol was taken to this 5ml of methyl paraben and propyl paraben was added and this mixture was added to carbopol. Grape seed oil extract was added under constant stirring to the carbopol mix until the gel consistency was obtained.

Test For Anti Oxidant And Anti Inflammatory Activity

Anti Oxidant Activity Test:

2,2diphenyl 1 picrylhydraazyl hydrate

DPPH assay was used to test the antioxidant activity of grape seed oil infused with silver nanoparticles. Grape seed oil silver nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mMTrisHCl buffer (pH 7.4) and incubated for 30 minutes.

Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT (Butylatedhydroxytoulune)was employed as control. The percentage of inhibition was determined from the following equation,
% inhibition= (Absorbance of control- Absorbance of test sample × 100)/Absorbance of control

Anti Inflammatory Activity Test:

Albumin denaturation assay

The anti-inflammatory activity for Grape seed oil gel was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations . 0.05 mL of Grape seed oil gel of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin(1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid.

These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO (dimethyl sulfoxide)is utilised as a control.

Percentage of protein denaturation was determined utilizing following equation,
% inhibition= (Absorbance of control- Absorbance of sample×100)/Absorbance of control

Statistics

All the data were analysed using the SPSS version 23.0 , Independent t test was done to assess the differences between the percentage of inhibition in the standard and GSO gel amongst all the five concentrations.

Results

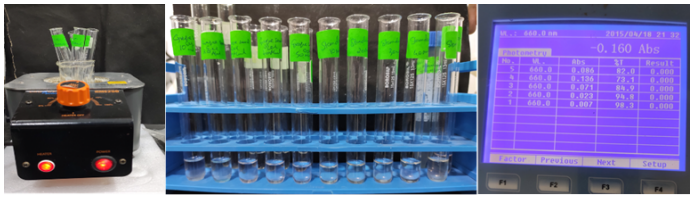
From the results obtained it was seen that GSO gel showed and retained both anti oxidant and anti inflammatoryactivity.It was observed that the anti oxidant activity of GSO gel was lesser than that of standard at all concentration but the differences are not statistically significant (p value 0.400)whereas in the experiment to test the anti inflammatory activity of GSO gel it as seen that the percentage of inhibition at all concentration except for the highest concentration was higher than that of the standard which is statistically significant (p value 0.045).

Discussion

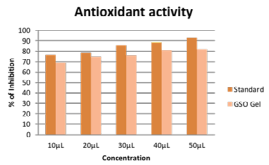
The study was conducted to assess whether the GSO gel infused with silver nanoparticles retained its anti oxidant and anti inflammatory activity . It is well known that components of vitisvinfera in common terms grapes are rich in antioxidants and has anti inflammatory activity because of the flavonoids and polyphenols present in them also this GSO extract is infused with silver nanoparticle and Silver metal has been widely used as a therapeutic agent for curing diseases. Silver nanoparticles (Ag NPs) have shown excellent bactericidal properties against a wide range of

Figure 1: Prepared gel

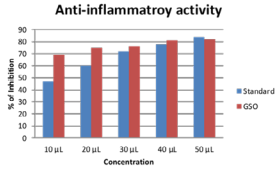
Figure 2: shows the anti-inflammatory activity in which two preparations were prepared, standard and GSO gel. The preparations were heated for 1 hour and to detect anti-inflammatory activity Uv-spectrometer with a wavelength range 660 was used.



Graph 1: The graph represents the results obtained from the experiment done to assess the antioxidant activity of grape seed oil gel.The X axis represents the percentage of inhibitions.
It is seen that the percentage of inhibition increases progressively as the concentration increases. At 50 mul the percentage of inhibition was 82% and the percentage of inhibition was 92% with that of standard at 50 mul concentration.



Graph 2: The graph represents the results obtained from the experiment done to assess the anti inflammatory activity of grape seed oil gel.The X axis represents the percentage of inhibitions.It is seen that the percentage of inhibition is higher in GSO gel when compared to the standard .At 10 mul the percentage of inhibition of GSO gel was 69% and the percentage of inhibition was 45% with that of standard at 10 mul concentration. At 50 mul the percentage of inhibition of GSO gel was 82% and the percentage of inhibition was 84% with that of standard at 50 mul concentration.



microorganisms[9,35,30].

From the results obtained it was seen that GSO gel shows both antioxidant and anti inflammatory activity. In the test for antioxidant activityit is seen that the percentage of inhibition increases progressively as the concentration increases. At 50 mul the percentage of inhibition was 82% and the percentage of inhibition was 92% with that of standard at 50 mul concentration. In the test for anti inflammatory activity of GSO gel it is seen that the percentage of inhibition is higher in GSO gel when compared to the standard .At 10 mul the percentage of inhibition of GSO gel was 69% and the percentage of inhibition was 45% with that of standard at 10 mul concentration. At 50 mul the percentage of inhibition of GSO gel was 82% and the percentage of inhibition was 84% with that of standard at 50 mul concentration.

Reactive oxygen species are important regulatory agents in the complex signaling network of cells. They play a major role in promoting cell growth and differentiation, adaptation to metabolic and physiological stresses, immune response as well as protection from pathogen invasion.

Flavonoids in grape seed oil also may act as indirect antioxidants by up-regulating antioxidant defence systems and increasing uric acid concentration in the plasma.

Therefore, because of their metabolic conversion in the human body, flavonoids generate large amounts of simple phenolic acids, which have significant effects in scavenging free radicals and improving the action of other antioxidants.

It has also been demonstrated that proanthocyanidins in grape seeds have high anti-inflammatory action, because they scavenge free radicals, prevent lipid peroxidation and inhibit formation of pro-inflammatory.

Studies done by Karami et al have proven that grape seed express anti inflammatory and antioxidant activity also Harbeoui et al concluded that Grape seed extract could be applied for the development of anti-inflammatory drugs.(Karami, Rahimi and Babaei, 2018) .Govindaraj et al have mentioned that Grape seed extract (GSE) is the richest source of proanthocyanidins and grape seeds have antioxidant, free radical scavenging, anticarcinogenicandanti inflammatory properties[25]. Clinical data have shown that procyanidin oligomers from grape seeds are 20 times more potent than vitamin C and 50 times more potent than vitamin E as antioxidant[36].

Jianmeiyu et al mentioned that grape seed polyphenols also inhibit some enzymes that catalyse the release of histamine, which is responsible for inflammation and allergies[1].

Prior studies have mentioned that Phenolics of grape seeds may help to inhibit enzyme systems that are responsible for the production of free radicals and that are associated with inflammatory reactions[22]. Procyanidins intervene in the synthesis and release of many sub-stances that promote inflammation, for example, histamine, serine protease, prostaglandins, and leukotrienes[27].

To our knowledge our study is the first to conduct an experiment to assess whether grape seed oil retains its properties when incorporated into gel medium. It is even that the antioxidant and anti inflammatory properties are still retained.

Conclusion

From the results obtained it is observed that the antioxidant and anti inflammatory properties of GSO gel has been retained and it is seen that the antioxidant and anti inflammatory activity increases as the concentration increases.Further trials could be conducted in animal models, before clinical trials.

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