Analysis of Antioxidant and Antiinflammatory Properties of Green Tea and Coffee Formulation - An Invitro Study

Amritha Sripoo.R[1], Uma Maheswari T.N[2], Rajeshkumar.S[3]
[1]postgraduate, Oral Medicine And Radiology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-6000077, Ph.No- 7373732669, Orcid- 0000-0002-5952-5755
Email: dramrithasripoo22@gmail.com
[2]professor And Head Of Admin, Department Of Oral Medicine And Radiology, Saveetha Dental College And Hospitals, Saveetha University, Chennai:6000077, Ph.No- 9840958339, Orcid- 0000-0002-2366-2336
Email: umamaheswaritn@saveetha.com
[3]NANOMEDICINE LAB, DEPARTMENT OF PHARMACOLOGY, SAVEETHA DENTAL College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai:6000077
Email: rajeshkumars.sdc@saveetha.com

Abstract

BACKGROUND Green tea from Camellia sinensis can be considered as an important dietary source of polyphenols, particularly flavonoids. Green tea contains a catechin called epigallocatechin-3-gallate (EGCG). Catechins are natural antioxidants that helps prevent cell damage and provides many other benefits.Coffee consists of the dried ripe seeds of Coffea arabica Linn which contains polyphenols that are attributed to a number of pharmacological activities that include antioxidant, antiinflammatory, immunomodulatory, anti-microbial, anti-cancer, cardioprotective and neuroprotective effects. **AIM** To assess the antioxidant and antiinflammatory activity of green tea and coffee for the formulation of in-situ gel in treating oral mucosal lesions. **MATERIALS AND METHODS** This study includes the usage of about 1g of green tea and 1 g of coffee mixed with 100 mL of distilled water and boiled for 15 minutes, filtered and again concentrated till 10mL. The antioxidant activity of green tea and coffee formulation is assessed by mixing it with DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) assay.The antiinflammatory activity of this formulation is assessed by using Bovine Serum Albumin assay. **RESULT** The spectrophotometer readings of antioxidant assay shows that 60% of green tea and coffee extracts have more absorption percentage when compared with standard.The anti-inflammatory assay has revealed that more than 90% of inhibition has been seen in 40µl &50µl concentrations of green tea and coffee when compared to other levels. **CONCLUSION** This study shows that the formulation of green tea and coffee produces good antioxidants when compared with many other commercially available antioxidants. It also contains good anti-inflammatory properties. Hence green tea and coffee can be used as safe alternatives instead of commercially available chemicals used for treatment of oral mucosal lesions.

Keywords: Green tea, coffee, Antioxidant, Anti inflammatory

1. Introduction

An antioxidant is a molecule that has the ability of slowing or preventing the oxidation of other molecular agents. Oxidation reactions produce free radicals that damage cells through chain reactions(1)(2). Antioxidants are the substances which significantly destroy the free radicals–reactive oxygen species responsible for degenerative diseases.Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease and fibrosis etc(3,4). Antioxidants play many vital functions in a cell and have many beneficial effects when present in foods(5). Certain fruits and vegetables containing antioxidants such as ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins play an important role for treating various diseases(1).Major plant antioxidants are the secondary metabolites of shikimic acid pathway and phenyl-propanoid metabolism that includes phenolics, coumarins, tannins, chalcone, flavonoid, etc. Flavonoids–flavones, flavanones, flavonols, isoflavones, anthocyanin, chalcone–also inhibit cytotoxic low density lipoproteins (LDL).Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness(6)(7)(6,8). Although initial studies suggested that antioxidant supplements might promote health(9). Inflammation is a normal protective response to tissue injury caused by physical trauma,

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noxious chemical or microbial agents(3)(10).The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers(11).Natural products have contributed significantly towards the development of modern medicine and they are effective as antioxidant,antimicrobial and antiinflammatory agents. Of late, traditional medicine is being re-evaluated worldwide, by extensive research on different plant species and their active therapeutic principles.The following are such plants with wide therapeutic benefits.

Tea, the most widely used beverage, is obtained from plant Camellia sinensis leaves, family theaceae(12). Polyphenols constitute the most interesting group of green tea leaf components, and in consequence, green tea can be considered an important dietary source of polyphenols, particularly flavonoids(13).The prominent flavonoid in tea is the flavan-3-ols, catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, and their fermentative products–theaflavins, thearubigin. Dry green and black tea leaves have comparable amounts of flavonoid and green tea contains most of catechin, while on fermentation catechin decreases but flavones, quercetin, kaempferol, and myricetin are not affected(1). It's loaded antioxidants have many health benefits, which may include:improved brain function,fat loss,protecting against cancer and lowering the risk of heart disease(14).

The seeds of botanical genus Coffea may be raw, whole, roasted or ground.The prepared drink through such coffee seeds is also called as coffee.Among 70 species of coffee,only three are cultivated.75% of the world's production of coffee is provided by Coffea arabica, about 25% by Coffea canephora, and less than 1% by Coffea liberica and others(15). The constituents of coffee mainly includes caffeine, tannin, fixed oil, carbohydrates and proteins.It contains 2-3% caffeine,3-5% tannins,13% proteins and 10-15% fixed oils.Caffeine is present as a salt of chlorogenic acid (CGA) in the coffee seeds.Also it contains oil and wax.In coffee pulp, condensed tannins are the major phenolic compounds, while in the seeds, phenolic compounds exist primarily as a family of esters formed between hydroxycinnamic acids and quinic acid, collectively recognized as chlorogenic acids (CGA).Coffee is a rich source of dietary antioxidants, and this property has led to the understanding that coffee is a major contributor to dietary antioxidant intake(16).Coffee is known to have positive health benefits,especially in chronic liver disease. Our team has extensive knowledge and research experience that has translated into high quality publications (17–31)

The materials used in this study includes extracted compounds containing 1g of green tea and 1g of coffee, these materials were acquired from authentic biomaterial sellers. The extracts were subjected to antioxidant testing using DPPH (2,diphenyl 1-picryl-hydrazyl-hydrate) assay.The extracts were subjected to antiinflammatory testing using Bovine Serum Albumin assay.

2. Materials and Methods

Materials used:

Antioxidant testing:

The preparation is done by taking a beaker of 10 ml of distilled water and 1 gm of green tea and coffee extract and mixed together, these extracts were then heated thoroughly until the solution is reduced to about 1mL of concentration, in order to reduce the water and increase the concentration of extraction.The solution containing green tea and coffee is subjected to 5 different concentrations of 10 µL, 20 µL, 30 µL, 40 µL, 50 µL in solution containing 1mL of DPPH, the solution is maintained at a room temperature for 10 mins, this is followed by boiling of the contents at 55 degree Celsius for 10 -15 mins , this solution is then subjected to spectrophotometry and analysed for inhibition levels.

Antiinflammatory testing:

The extracts of green tea and coffee are subjected to Bovine serum albumin assay (BSA). The solution containing green tea and coffee formulations are taken in 5 different concentrations of 10 µL, 20 µL, 30 µL, 40 µL, 50 µL in solution containing 2mL of BSA, the solution is maintained at a room temperature for 10 mins, this is followed by boiling of the contents at 55 degrees Celsius for 10 -15 mins, this solution is then subjected to spectrophotometry for inhibition level analysis.

3. Result

The results of this study has shown a better inhibition percentage in antioxidant activity. The antioxidant activity testing was done in various levels of green tea and coffee extracts as 10 µL, 20 µL, 30 µL, 40 µL and 50 µL in a test tube containing 1ml of DPPH [Figure 1]. The spectrophotometer showed readings with antioxidant activity assay of green tea and coffee extracts. The spectrophotometry readings of antioxidant assay show that of green tea and coffee extracts have more absorption percentage of about 60%. The antiinflammatory activity done in various levels of green tea and coffee extracts as 10 µL, 20 µL, 30 µL, 40 µL and 50 µL in a test tube containing 2ml of BSA[Figure 1] ,shows that the formulation of green tea and coffee has a better Inhibition action in anti-inflammatory assay analysis. Spectrophotometry readings of anti-inflammatory assay have revealed that formulation of green tea and coffee of about 10 ml has given an absorption percentage of about 80%when compared with standard. The anti-inflammatory assay has revealed that more than 90% of inhibition percentage has been seen in 40µl &50µl concentrations of green tea and coffee when compared to other levels.

4. Discussion

Tea has antioxidants called catechins, which reduce inflammation. Epigallocatechin gallate (EGCG), is the most powerful and active component of green tea. Other teas have this effect too, but green tea has the most benefits.EGCG can reduce the inflammatory response associated with local tissue injuries. EGCG effectively alleviates cellular damage by lowering the inflammatory reaction and reducing the lipid peroxidation and NO generated radicals leading to the oxidative stress. Green tea is a dietary supplement in the prevention of cardiovascular diseases in which oxidative stress and proinflammation are the principal causes(13).A concentration-dependent inhibition of protein (albumin) denaturation was exhibited by both the green tea and black tea extracts and possessed a marked anti-inflammatory effect against the denaturation of protein, in vitro. Green tea was found to be more active than black tea, plausibly due to the higher flavonoid contents of green tea(32). Tea flavonoids are potent antioxidants, absorbed from the gut after consumption.Tea consumption leads to significant increase of antioxidant in blood.Beneficial effects of increased antioxidant capacity in the body may be the reduction of oxidative damage to important biomolecules.The scientific support is strongest for the protection of DNA from oxidative damage after black tea or green tea consumption(12). From the past research, it was already proved that the phenolic content is directly responsible for reduction of oxidative stress and the antioxidant potential of the plant extract depends on the presence of phenolic compounds and flavonoids.(1)Hence,green tea contains good antioxidant and antiinflammatory properties.

C. arabica and C. robusta are the two most commonly grown coffee bean types.All coffee plants are classified in the large family Rubiaceae.Roasted coffee is a complex mixture of over 1000 bioactive compounds(33),some with potentially therapeutic anti-inflammatory, antioxidant, antifibrotic, or anticancer effects that provide biological plausibility for recent epidemiological associations. Medicinally, caffeine is used as a CNS stimulant, usually combined with another therapeutic agent and in analgesic preparations(34,35).Caffeine has also been proposed as the component of coffee linked to the increased risk in women, with potential influence on calcium absorption and bone mineral density.Researches has explored the associations between coffee as an exposure and a range of outcomes including all-cause mortality, cancer, and diseases of the cardiovascular, metabolic, neurological, musculoskeletal, gastrointestinal, and liver systems, as well as outcomes associated with pregnancy.Previous studies have consistently found that long-term coffee consumption is associated with a lower risk of Parkinson's disease(36).Coffee consumption was consistently associated with a lower risk of mortality from all causes of cardiovascular disease, coronary heart disease, and stroke in a non-linear relation, with summary estimates indicating largest reduction in relative risk at three cups a day(37).A meta-analysis showed a lower incidence of cancer for high versus low coffee consumption(16).High versus low coffee consumption was associated with a lower risk of prostate cancer(38), endometrial cancer(39), melanoma(39,40),oral cancer(38), leukaemia(16), non-melanoma skin cancer(41),and liver cancer(42) there were also significant linear dose-response relations indicating benefit(36).

Further,Regularly drinking green tea can protect against cavities,gum disease and bad breath, according to a 2016 study that compiled research on the beverage's oral health effects. The study indicated that green tea may reduce oral bacteria which, in turn, can promote the health of teeth and gums(34).Oral cavity oxidative stress and inflammation, consequent to cigarette smoking and cigarettes deleterious compounds nicotine and acrolein, may be reduced in the presence of green tea polyphenols(34). Generally, green tea defends healthy cells from malignant transformation and locally has the ability to induce apoptosis in oral cancer cells.Thus researchers noted a significantly lower risk of oral cancer among individuals who drank green tea.In a previous study,Zhang et al. revealed that tea extract reduced a-amylase activity in human saliva. Therefore, tea consumption is likely to be an anticariogenic agent which lessens the cariogenic potential of starch containing foods like crackers and cakes. It might lead to less maltose release that causes mineral depletion from tooth enamel(43). Coffee beverages, additionally to caffeine, contains a variety of antioxidant and anti-mutagen agents including phenolic derivatives (such as chlorogenic acid and polyphenol caffeic acid) and diterpenes (such as cafestol and kahweol), that could act as carcinogenic detoxifying agents on oral and pharyngeal mucosa (44).Coffee consumption appears to have a protective benefit in oral cancer(45).Tavani et al found that different coffee beverages (caffeinated, decaffeinated and coffee) have different effects on oral cancer risk.

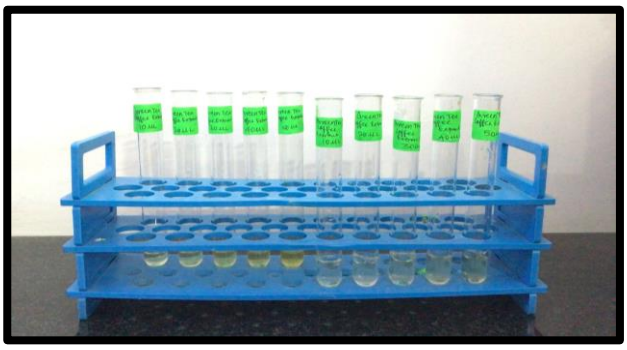

Fig 1: Shows antioxidant and antiinflammatory testing using various levels of green tea and coffee extracts: 10 µL, 20 µL, 30 µL, 40 µL and 50 µL extracts in test tube containing 1ml of DPPH and 10 µL, 20 µL, 30 µL, 40 µL and 50 µL extracts in test tube containing 2ml of BSA respectively.

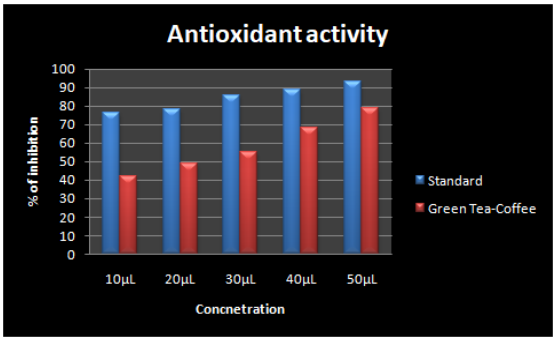

Fig.2: Shows percentage of inhibition of antioxidant assay seen in levels of 10 µL, 20 µL, 30 µL, 40 µL and 50 µL concentrations of green tea and Coffee formulations.

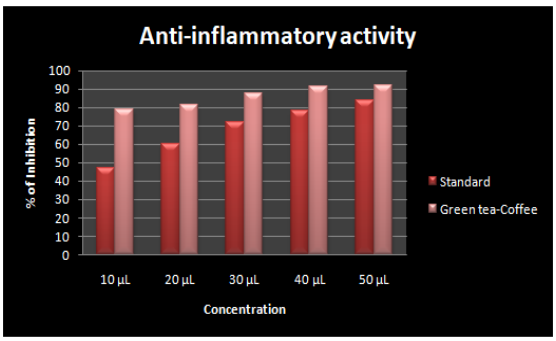

Fig.3: Represents the percentage of inhibition in anti-inflammatory assay seen in levels of 10µl, 20 µL, 30 µL, 40 µL and 50 µL concentrations of green tea and Coffee formulations.

5. Conclusion

The present study showed significant antioxidant properties.This combination of extract also have a better antiinflammatory effect hence this study concludes that combination of green tea and coffee formulation has better antioxidant and antiinflammatory properties and in future this can also be used as in-situ gel in management of oral mucosal lesions.

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