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Anti Inflammatory and Antioxidant Activity of Stevia and Peppermint Herbal Formulation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: It is important to counteract the oxidative stress and inflammatory burden occurring in the body. The use of herbal components obtained via green synthesis, such as Stevia and peppermint which are alcohol-free and comprise relatively of less chemical components and have been therapeutically found effective for antidiabetic properties can prove to be more efficient and biocompatible to tackle the same.
Aim: The aim of the study was to evaluate the anti-inflammatory and antioxidant properties of a herbal formulation of stevia rebaudiana and peppermint at varying concentrations.
Materials and Methods: The study was performed as an in vitro study under a laboratory setting. Synthesis of the herbal formulation was performed using stevia and peppermint in the lab. Subsequently the formulation was tested for its anti-inflammatory activity using the protein denaturation assay and antioxidant activity using the DPPH Assay method at various concentrations. The obtained values were compared with that of the known standard. The statistical analysis was done using IBM SPSS software analysis version 23.

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Results: Anti inflammatory property of the Steviaa and peppermint herbal formulation was significantly less at 10µL concentration but at higher concentrations it was comparable to the standard. Regaling the antioxidant property, the herbal formulation showed a concentration dependent increase from lower concentration to higher concentration but at all levels it was significantly lesser than the standard.
($p<0.05$)
Conclusion: The herbal formulation of Stevia and peppermint is a potent anti-inflammatory agent. It possesses antioxidant properties, however which are not as effective as the standard.

Keywords: Stevia; peppermint; anti-inflammatory; antioxidant; green synthesis; oxidative stress; free radicals.

1. INTRODUCTION

Inflammation is a complex reaction to injurious agents and includes vascular responses, migration and activation of leukocytes. Inflammation can be acute or chronic. Inflammation is terminated when the invaders are eliminated and the secreted mediators are removed [1]. Inflammation can also lead to the development of many systemic illnesses when it persists over a long period of time such as diabetes, allergic hypersensitivity reactions etc. In the oral cavity pertaining to the periodontium, periodontitis always begins with inflammation of the gingiva, causing gingivitis and subsequently proceeding to periodontitis. Reciprocal effects of periodontal diseases are potential factors modifying the severity in the progression of systemic diseases [2,3]. Virtually, almost all inflammatory diseases lead to increased oxidative stress. This in turn can trigger more damage to the tissues, not excluding the gingival tissue and, thus, worsening periodontitis [4].

The physiological and pathological changes in the human body depend on the free radical and reactive oxygen species (ROS) interactions to maintain the normal cellular activities [5]. The free radicals when not balanced have deleterious effects on the organs of the body and can be the trigger for rheumatoid arthritis, stroke, liver diseases and various cancers. In aerobic organisms the imbalance between the ROS generation and the antioxidants level leads to oxidative stress and cellular degeneration. Oxidative stress is involved in the pathogenesis of many diseases besides periodontitis for in the pathogenesis of periodontal diseases, the increased polymorph neutrophils count and activity cause a high rate of ROS release. This leads to increased oxidative stress in periodontal tissues [6]. Periodontal tissues require adequate levels of antioxidants to prevent tissue damage caused by reactive oxygen species. Antioxidants can counter the formation of free radicals and prevent the free radical damage from occurring by donating electrons [7]. Chronic inflammation and oxidative stress are two important factors which contribute to many of the oral diseases including cancer [8]. Non steroidal anti-inflammatory agents are effective in controlling the inflammation but the long term usage of the same is with the added side effects. Many plant products have been tried in the past with beneficial therapeutic effects and less side effects [9,10].

Stevia rebaudiana Bertoni, an ancient perennial shrub, produces diterpene glycosides that are low calorie sweeteners [11]. Besides having therapeutic properties, they contain a high level of sweetening compounds, such as steviol glycosides, the other components include, vitamin C and amino acids, phenolic components, flavonoids, which are thought to be efficient for antioxidant, antimicrobial and antifungal activities [12,13]. Stevia is also known to possess many other therapeutic properties for cardiac and dermatological conditions [14]. The consumption of Stevia can induce various side effects too in humans such as nausea, dizziness and fatigue hence it is important that it is uptake in prescribed dosage.

Peppermint is a hybrid plant that has been proven to help as a cure for skin problems, cold and flu, nausea and headaches [15]. Peppermint oil is widely used as a flavouring and sweetening agent in dentistry as dentifrices, mouthwashes, toothpastes etc. it is said to possess antimicrobial properties and ease the digestive system [16]. Peppermint is a good source of manganese, copper and vitamin C [17]. Mint also contains vitamins and minerals including vitamins A, B-6, C, E, and K, beta carotene, folate and riboflavin and the minerals calcium, iron, potassium, magnesium and manganese. Fresh mint is a powerful antioxidant [18].

Our team has a plethora of research and knowledge that has resulted in high-quality publications [19-38].

The aim of the study was to assess the antioxidant and anti-inflammatory properties of Stevia and peppermint herbal formulation at various concentrations and compare the efficacy of the same in an in vitro model.

2. MATERIALS AND METHODS

2.1 Preparation of Extract

Equal weights of powdered Stevia and peppermint were taken and made up to a solution of 100mL using distilled water. The solution was boiled, cooled down and filtered to obtain the extract. The extract was concentrated to 10% and transferred to an Eppendroff tube.

2.2 Anti-inflammatory Activity

The anti-inflammatory activity for Stevia and peppermint herbal formulation was tested by the following method :

0.05mL of Stevia and peppermint herbal formulation at various fixation (10µL, 20µL, 30µL, 40µL, 50µL), was added to the 0.45mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity f 1N HCl. These samples were incubated at room temperature for 20 mins and then at 55°C in a water bath for 30 mins. The samples were cooled and the absorbance estimated spectrophotometrically at 660nm. Diclofenac sodium was used as the standard. Dimethyl sulfoxide is used as a control. Percentage of protein denaturation was determined using the following equation:

$$\%\ protein\ denaturation = \frac{[Absorbance\ of\ control\ -\ Absorbance\ of\ sample]}{Absorbance\ of\ Control} x\ 100$$

2.3 Antioxidant Activity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay was used to test the antioxidant activity of stevia and peppermint herbal formulation. 3 tubes of each concentration ranging from 10µL to 50µL, with difference of 10µL was mixed with 1mL of 0.1mM of DPPH in methanol and 450µL of 50mM of tris HCl buffer (pH=7.4) and incubated for 30 mins. Later the reduction in quantity of DPPH free radicals was assessed dependent on the absorbance at 517nm. Butylated hydroxytoluene was employed as a control. Percentage of inhibition was determined using the following equation:

$$\%\ inhibition = \frac{[Absorbance\ of\ control\ -\ Absorbance\ of\ sample]}{Absorbance\ of\ Control} x\ 100$$

2.4 Statistical Analysis

The obtained data was sorted in MS Excel and statistically analysed by performing the unpaired t-test for the comparison with the standard and one way- ANOVA followed by Tukey's post hoc. Statistics for the comparison between different concentrations using IBM SPSS version 23. Results depicted in graphs and tabulations.

3. RESULTS

The analysed data yielded the following results :

3.1 Anti-inflammatory Activity

The results are as depicted in the graph (Fig. 1), the anti-inflammatory activity of the standard was significantly greater than that of the herbal formulation at 10µL concentration, however with increase in concentration there was an increase in the anti-inflammatory activity of the herbal formulation. Anti inflammatory effect was significantly more at 20µL compared to the anti inflammatory activity of the standard at that concentration ($p<0.05$). The anti-inflammatory activity of the standard and herbal formulation was found to be similar with further increase in concentration.

One way ANOVA analysis followed by post hoc test compared the anti-inflammatory activity of the herbal formulations at varied concentrations (Table 1) and it revealed that among the various concentrations of the samples tested there was a concentration dependent change in anti-inflammatory activity from 10µL to 20µL and thereafter there was no significant change as observed in this study.

3.2 Antioxidant Activity

The results of the DPPH assay (Fig. 2) revealed that at all concentrations from 10µL to 50µL, the herbal formulation had significantly less antioxidant capacity as compared to the standard ($p<0.05$) (Fig. 2). There was a concentration dependent increase in antioxidant

effect but as the concentration increased from 20µL to 50µL the difference in the antioxidant capacity between the herbal formulation was comparatively less as compared to 10 to 20µL (Fig. 2).

One way ANOVA followed by post hoc analysis, revealed that there was a concentration dependent increase in the antioxidant capacity of herbal formulation. Antioxidant capacity was least at 10µL concentration and it was more than doubled at 20 µLwhich was statistically significant as observed in this study. The antioxidant activity was found to be increased significantly with increase in concentration of the herbal mouthwash which was statistically significant, however it was not as effective as the standard.

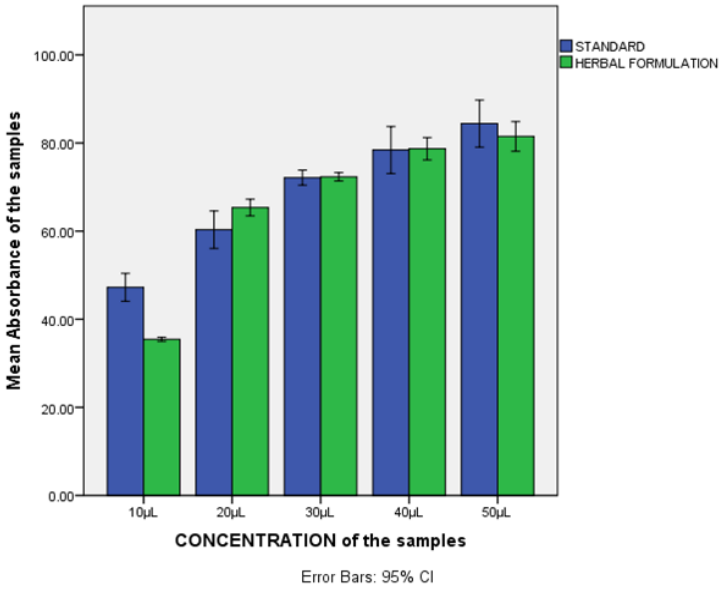

Fig. 1. Graph depicts the anti-inflammatory activity of Stevia and peppermint herbal formulation compared to the standard. The Y-axis depicts the mean absorbance values of the samples. The X-axis denotes the various concentrations. The blue bar depicts the standard while the green bar depicts the herbal formulation. Error percentage has been set upto 95%. The mean absorbance was almost similar to the standard at higher concentrations (p>0.05, unpaired t test), there was a significant increase in the absorbance value with increase in concentrations (p<0.05, one way anova followed by post hoc analysis)

Table 1. Table depicts the statistical comparison of anti-inflammatory activity at various concentrations using one way ANOVA analysis followed by post hoc. (p<0.05)

One way ANOVA Anti-inflammatory Activity Statistical Analysis		
Concentration (I)	**Concentration (J)**	**Significance (p<0.05)**
10µL	20µL	0.035*
	30µL	0.007*
	40µL	0.003*
	50µL	0.002*
20µL	30µL	0.687
	40µL	0.131
	50µL	0.046*
30µL	40µL	1.000
	50µL	0.514
40µL	50µL	1.000

**(p<0.05)*

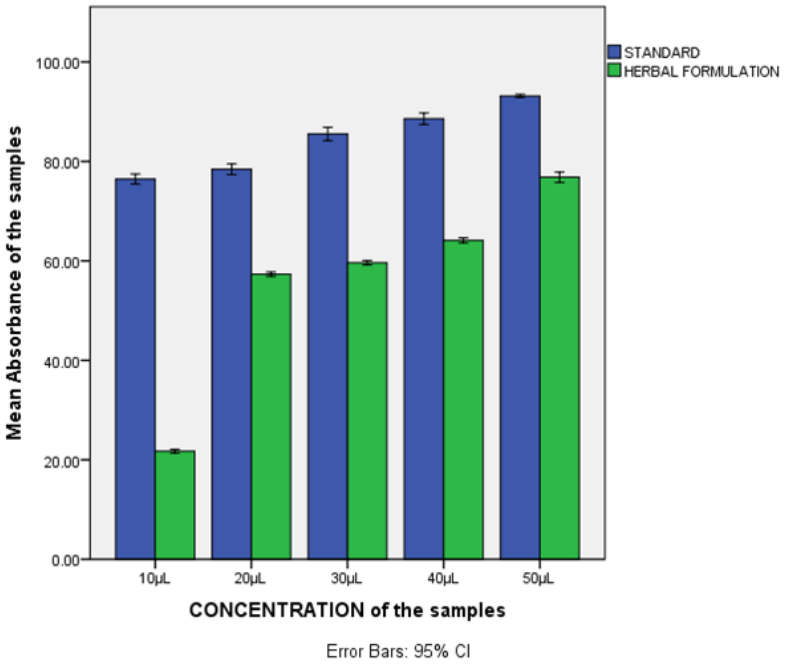

Fig. 2. Graph depicts the antioxidant activity of Stevia and peppermint herbal formulation compared to the standard. The Y-axis depicts the mean absorbance values of the samples. The X-axis denotes the various concentrations. The blue bar depicts the standard while the green bar depicts the herbal formulation. Herbal formulation had significantly less antioxidant activity as compared to standard at all concentrations (p>0.05, unpaired t test , Error percentage has been set upto 95%) but there was a significant increase in the absorbance value with increase in concentrations (p<0.05, one way anova followed by post hoc analysis)

Table 2. Table depicts the statistical comparison of antioxidant activity at various concentrations using one way ANOVA analysis followed by post hoc. (p<0.05)

One way ANOVA antioxidant activity statistical analysis		
Concentration (I)	**Concentration (J)**	**Significance (p value)**
10µL	20µL	0.000*
	30µL	0.000*
	40µL	0.000*
	50µL	0.000*
20µL	30µL	0.000*
	40µL	0.000*
	50µL	0.000*
30µL	40µL	0.000*
	50µL	0.000*
40µL	50µL	0.000*

**(p<0.05)*

4. DISCUSSION

The study aimed to assess whether there was any anti-inflammatory and antioxidant activity for the prepared Stevia and peppermint herbal formulation and to test its efficacy with the known standards.The five different concentrations of the plant samples were analysed for its antioxidant and anti-inflammatory activity and it was compared with the same concentration of the standard drugs. The concentration dependent assay clearly showed the activity of the prepared herbal formulation. The results revealed that the tested formulation had both anti-inflammatory and antioxidant capacity. In comparison with the known Non steroidal anti inflammatory drug Diclofenac sodium, even though the anti inflammatory property of the herbal formulation

was significantly less at 10µL concentration but at higher concentrations it was comparable to the standard. Regaling the antioxidant property, even though the herbal formulation showed a concentration dependent increase from lower concentration to higher concentration at all levels it was significantly lesser than the standard.

The anti-inflammatory activity of the herbal extract is conferred by compounds present in the leaves of stevia including stevioside, rebaudioside and isosteviol. The anti-inflammatory activity of the herbal extract was found to be as effective as the standard as observed in this study, however there exists a varying efficacy of the herbal formulation with change in gradient. This could be owing to the various components present in peppermint and stevia and their individual properties. Previous studies have also cited facts which prove that stevia possesses anti-inflammatory activity as it plays an important role in the body by intervening in the I-Kappa-B kinases and Kappa-B signalling pathways which are basically inflammatory agents [39–41].

In this study it was observed that although the antioxidant activity of the herbal formulation was not better than that of the standard, with increasing concentrations there was an increase in the antioxidant activity of the herbal formulation significantly observed from 10µL to 20µL and with increasing concentration of the herbal formulation the effectiveness of the standard was almost the same. The antioxidant activity of the herbal formulation is less compared to the standard, this might be due to the inclusion of peppermint along with stevia in this composition of herbal formulation. It has been reported that the free radical scavenging activity of *S. rebaudiana* extract increased with increasing concentrations. Some authors indicate that *S. rebaudiana* extract exhibited the ability to quench the DPPH radical, which indicates that it was a good antioxidant with radical scavenging activity [42]. The antioxidant activity of Stevia is contributed by the phenolic compounds and flavonoids present in the leaves of the plant predominantly. Previous studies demonstrated prominent radical scavenging and Fe3+ reducing activity in chemical-based assays [43,44]. In comparison with native and Scotch spearmint essential oil, peppermint essential oil had the lowest ($p< 0.05$) half maximal effective concentration in DPPH and TEAC assays and higher efficacy in the reducing power assay [45,46]. Previous literature is in concordance with our findings [47-60]. The debatable point with regard to stevia as reported in earlier studies is that there are some side effects which include nausea, dizziness, headache, fatigue, bloating and diarrhoea [61]. These tend to occur in dose dependent consumption of stevia [62]. Peppermint on the other hand is known widely for its ability to ease the digestive system, aid in nausea and dizziness, hence acts as a perfect blend masking the effect of stevia [63]. The results of this study confirm that Stevia and peppermint herbal formulation is a potent anti-inflammatory agent with moderate antioxidant capacity, but these results have to be validated with further cell culture studies and in vivo studies to recommend the same for clinical usage.

5. CONCLUSION

In the present work, stevia and peppermint herbal formulation was found to have potent anti-inflammatory and anti-oxidant activity. This potential effect of the herbal formulation paves the way to use it as an effective agent in treating periodontitis and also its enormous role in future dental field.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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