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RESEARCH ARTICLE

Evaluation of the Antioxidant Interactions Between Green Tea Polyphenols and Nonsteroidal Anti-inflammatory Drugs

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Abstract:

Introduction:

The antioxidant interactions between the commonly used pharmaceuticals (diclofenac, ibuprofen and naproxen) and green tea polyphenols were evaluated.

Methods:

The antioxidant properties of the mixtures were evaluated by a scavenging effect on the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical.

Results:

The mixtures contained green tea extract and each drug exhibited lower antioxidant activity than the mathematical sum of the results obtained for individual components showing antagonistic effect. The results were obtained using isobolographic analysis and interaction factors also suggested the antagonistic type of interaction. Only when the concentration of the green tea infusion was relatively high (in comparison to the drug), an additive effect could be concluded.

Conclusion:

The high concentration of green tea infusion in comparison to the drug should be used in developing the new formulations as it can help in the therapy due to their antioxidant properties.

Keywords: Green tea polyphenols, Nonsteroidal anti-inflammatory drugs, Isobolographic analysis, Interaction factor, Antioxidant, DPPH.

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1. INTRODUCTION

Tea is the most consumed flavoured functional beverage in the world. Green tea is prepared from steamed leaves of *Camellia sinensis* in the absence of fermentation and it contains carbohydrates, vitamins and most importantly polyphenols, which possess high antioxidant activity [1]. Catechins are the major polyphenolic compounds in tea leaves. Scientific studies have indicated the effects of green tea consumption for improving human health and in the reduction of risk in severe diseases, particularly neurodegenerative and cardiovascular diseases [1 - 3]. These effects are linked with the ability of polyphenols to scavenging of free radicals, chelation of metal ions (such as iron or copper) that accelerated oxidation processes, quenching of singlet oxygen and act as reducing agents [4, 5].

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent an effective pain treatment option and they are medicated for numerous diseases including arthritis, osteoarthritis and musculoskeletal diseases [6]. They are one of the most sold therapeutic agents worldwide due to their availability over-the-counter. The therapeutic effect of NSAIDs is generally based on the inhibition of the cyclooxygenase pathway and blocking the production of prostaglandins, which are responsible for the induction of inflammation, pain and fever [7, 8]. Several studies have also demonstrated that NSAIDs are effective in the prevention and treatment of some cancers [6, 9]. However, prolonged use and overdose of these drugs can lead to peptic ulcer, intestinal perforation, bleeding, hepatotoxicity, liver and kidney damage [6, 10, 11].

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The interaction between the analgesic drugs and herbal infusions containing great amounts of catechins, such as green tea, can lead to an increase of anti-inflammatory activity as well as the added protection of kidney and liver from NSAIDs damage [6]. Several reports show synergistic or antagonistic interactions of green tea or catechins with some drugs [12 - 16].

This paper presents data on the influence of the most consumed NSAIDs such as diclofenac, ibuprofen and naproxen on the antioxidant activity of the Green Tea Extract (GTE). For this purpose, the antioxidant properties were evaluated by the scavenging effect on the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical. In particular, the antioxidant activity of the mixture of GTE and a given drug was compared with that of the single components measured individually. The potential antagonistic, additive or synergistic effects were also evaluated using the isobolographic analysis and interaction factors [16 - 18]. The results of this study can be useful in designing new formulations with the addition of polyphenolic compounds, which can prevent or minimize the negative impact of drugs on the human body.

2. MATERIALS AND METHODS

2.1. Reagents

Commercial standards of diclofenac, naproxen, ibuprofen and green tea extract Polyphenon 60 (CAS No. 138988-88-2), as well as the other chemicals, were purchased from Sigma (Steinheim, Germany). Ultra pure water from the Milli-Q system (Millipore, Bedford, MA, USA) with the electrical resistivity of 18 MΩ/cm was used in all experiments.

2.2. Preparation of Mixtures for Isobolographic Analysis

Green tea standards, as well as solutions of pharmaceuticals, were prepared just before the mixture preparation. For every tested substance, linear calibration curves were obtained to check for the linearity between the sample concentration and the observed effect in the DPPH assay. Mixtures of the following concentration ratios were prepared: 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, and 1:10. Five independent samples (different concentrations of analytes but the same concentration ratio between them) were analyzed for every measured ratio. Each sample was analyzed in triplicate.

2.3. Scavenging Ability on DPPH Method

For DPPH assay, 0.1 ml of the sample (containing individual compounds or their mixture) was added to 2.4 ml of DPPH solution (3.0×10^{-5} M) in methanol. After 30 min, absorbance was measured at λ = 539 nm ((UV-Vis spectrophotometer, Lambda 20 Perkin Elmer). The results were expressed in terms of the EC_{50} value or trolox equivalent (TRE, in mmol/L). Analyses were run in triplicates.

2.4. Statistical Analysis

The results are from at least three independent experiments and are presented with average ± standard deviation. One-way ANOVA and Tukey tests were used to determine the difference of means. Significance was defined at P values < 0.05.

3. RESULTS AND DISCUSSION

The study was conducted to determine whether the addition of NSAIDs affects the antioxidant capacity of green tea extract. Three widely used painkillers such as naproxen, ibuprofen and diclofenac were involved in the study. Their structures are presented in Fig. (**1**). The method using DPPH radicals is one of the most frequently employed methods to test the ability of the sample to scavenge free radicals, where a hydrogen transfer reaction is involved [19].

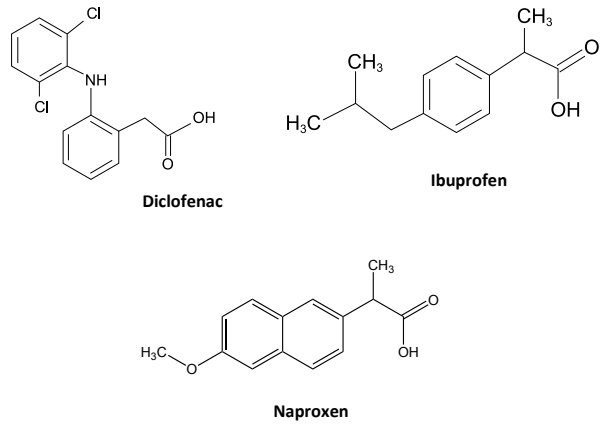

Fig. (1). Structures of the studied NSAIDs.

The antioxidant activities (expressed as Trolox equivalent, TRE) obtained experimentally for a single component and their mixtures were compared with theoretical values calculated by adding up the effects of both individual components analysed separately. All comparisons were based on the same concentration (10 mg/L) of the components. The obtained results are presented in Fig. (**2**). It can be seen that the mixtures contained green tea extract and each drug exhibited lower antioxidant activity than the mathematical sum of the results obtained for individual components, showing antagonistic interactions. The main difference was observed for the mixture of GTE and diclofenac. The solutions of studied NSAIDs exhibited low antioxidant activity in DPPH assay and their TRE values increased in the following order *i.e*. naproxen < diclofenac < ibuprofen. Interestingly, Končič *et al*. [20] reported that hydroxyamic acid derivative of diclofenac was a better radical scavenger in the DPPH assay than the same derivative of ibuprofen. Several hypotheses have been postulated to explain synergy or antagonism in the antioxidant response of food-drug mixtures and these effects depend on the polarity of the interacting molecules, reaction rates and the effective concentrations of the compounds [21 - 23].

The antioxidant interactions of green tea extract and studied drugs with their different ratios were also evaluated using isobolographic analysis [17]. This method requires a linear relationship between an activity and concentration of a compound, and is independent of the mechanism of activity. The antioxidant activity was expressed in terms of the EC_{50} value, *i.e*., the concentration (in mg/L) required to scavenge 50% of the DPPH radicals, calculated by linear regression

analysis [24]. Five independent samples (different concentrations of GTE and a given drug, but the same concentration ratio between them) were analyzed for the following measured ratios: 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, and the EC_{50} factors for each mixture were calculated. The individual EC_{50} factors for green tea extract and drugs were plotted on the x- and y-axes, respectively. The line between these two points is a theoretical additive line (isobola). The points lying below it represent the synergistic effect, while the points lying above the isobola indicate that the effect between substances is antagonistic. The obtained isobolograms are presented in Fig. (**2**). The results indicated that antiradical scavenges included GTE and all drug mixtures acted mainly antagonistically. This confirmed the results presented in Fig. (**2**), although they were obtained only for one ratio of the components.

The type of interaction determined on the basis of isobolographic analysis was confirmed by the values of Interaction Factor (IF) presented in Table **1**. They were calculated according to the following equation; $IF = C_A + C_B$, where C_A and C_B are the concentrations of a given component in the mixture divided by its concentration having the same effect as the mixture [18]. The value of IF higher than 1 indicates a synergistic effect; a value below 1 indicates antagonism, while a value equal to 1 means additive interactions. The calculated interaction factors were higher than 1, particularly at a higher excess of the studied drugs in comparison to GTE, implying the antagonistic effect. A similar antagonistic type of interaction effect in the DPPH assay was observed between green tea polyphenols and acetaminophen [16] (Fig. **3**). When the concentration of the green tea infusion was relatively high (in comparison to the drug), IF values were close to 1. Such combination seems to be reasonable in developing a new drug or formulation as it can prevent the side effects caused by the pharmaceutical and help in the therapy due to its antioxidant properties. However, some caution is advised in the consumption of significant amounts of green tea extract because of the reported hepatotoxicity [1, 15].

Table 1. Interaction factors for studied drugs and green tea extract mixtures at different concentration ratios.

Ratio GTE:Drug	Naproxen	Ibuprofen	Diclofenac
10:1	1.25 ± 0.068	1.07 ± 0.057	1.87 ± 0.093
5:1	1.05 ± 0.065	1.07 ± 0.062	1.20 ± 0.061
2:1	1.31 ±0.071	1.18 ± 0.060	1.17 ± 0.058
1:1	1.04 ± 0.065	1.02 ± 0.059	1.20 ± 0.066
1:2	1.67 ± 0.081	1.14 ± 0.074	1.60 ± 0.078
1:5	2.93 ± 0.119	2.24 ± 0.112	7.26 ± 0.390
1:10	4.03 ± 0.208	2.30 ± 0.179	4.86 ± 0.210

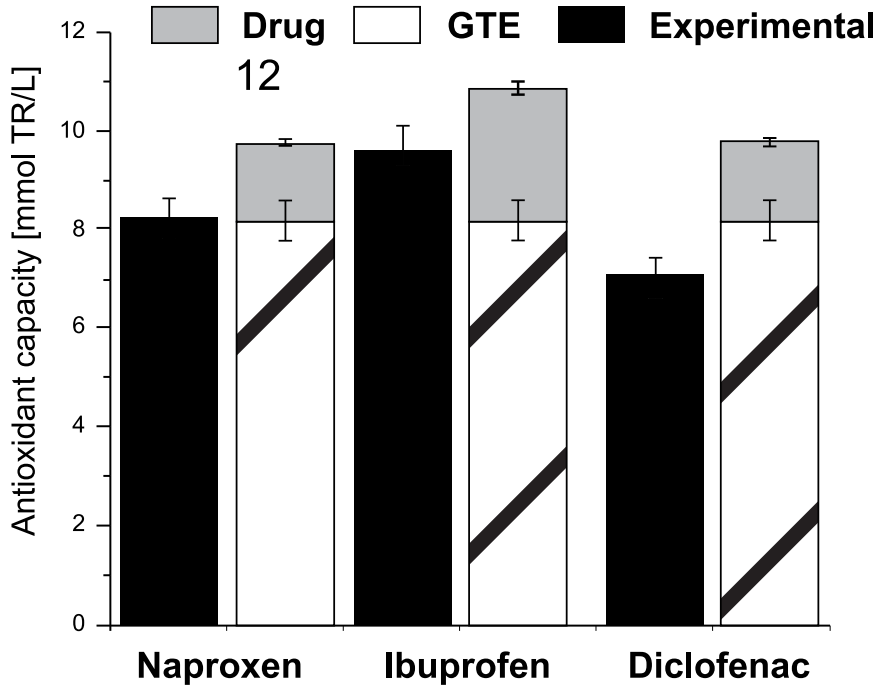

Fig. (2). Antioxidant activity of the mixtures containing a given drug and green tea extract in the DPPH assay. Each compound at a concentration of 10 mg/L.

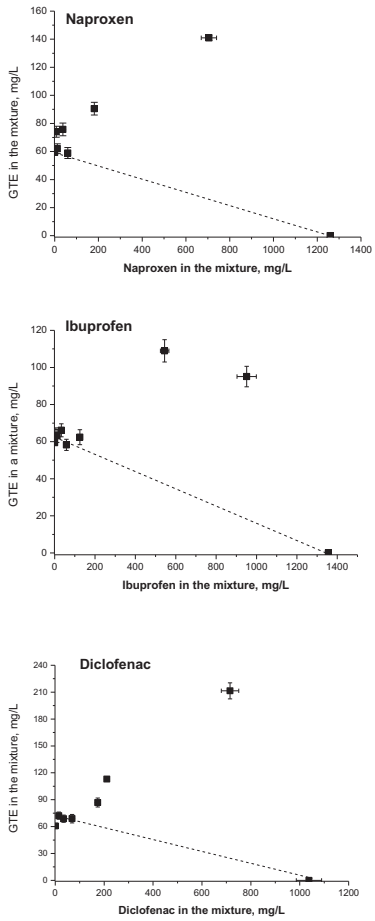

Fig. (3). Isobolograms for green tea polyphenols and pharmaceuticals in the DPPH assay. The EC_{50} values for a given pharmaceutical and GTE are placed on the graph, on *X*- and *Y*-axes, respectively. The isoboles of additivity are shown as dashed lines drawn between the appropriate EC_{50} values. The solid points depict the experimental EC_{50} values of the mixtures for their fixed-ratio combination.

CONCLUSION

In this study, the antioxidant interactions between the commonly used NSAIDs (naproxen, ibuprofen and diclofenac) and green tea polyphenols were investigated using the DPPH assay. The obtained results showed that the effect between the pharmaceutical and polyphenols from green tea extract is antagonistic. However, future studies are needed with other *in vitro* assays measuring different aspects of the behavior of antioxidants. It should be helpful to explain the interaction mechanisms of drug-green tea interactions.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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