Short communication **Bimonthly published scientific journal**

INTERNATIONAL JOURNAL of Indigenous Herbs and Drugs

ISSN-2456-7345

Evaluation of anti-inflammatory activity of hydroalcoholic extract of black tea leaves *in-vitro*

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Abstract

Objective: The objective of present investigation was to determine the activity of hydroalcoholic extract of tea leaves against protein denaturation and inflammation.

Methods: The method employed for the investigation comprised of a reaction mixture containing egg albumin, phosphate buffer and varying concentrations of hydroalcoholic extract. Individual mixtures were incubated at 37 ºC for 15 min followed by heating at 70 ºC for 5 min. Absorbance of different mixtures was measured at 660 nm. Indomethacin was taken as standard drug. Percent inhibition was calculated.

Results: Percentage inhibition of hydroalcoholic extract and indomethacin was found to be increasing with increase in their respective concentrations (250-1250 µg/ml).

Conclusion: The present study provides useful information about the anti-inflammatory activity of hydroalcoholic extract of black tea leaves. Hydroalcoholic extract showed significant decrease in inflammation, although the action was found to be less than that of standard drug.

Keywords: Inflammation, Indomethacin, Hydroalcoholic Extract, Protein Denaturation, Percent Inhibition

Inflammation is the body's challenge in the form of immune response to produce self-protection against the damages like cell destruction, irritability, infection etc. An inflammatory response consists of several physiological processes, all of which are triggered by the release of pharmacologically active substances such as histamine and the chemical mediators released from injured tissues and migratory cells. Whatever may be cause for inflammation, all are characterized by four cardinal signs viz. redness, swelling, heat and pain. The goal of treatment for inflammation is to reduce or prevent the production of inflammatory agents that trigger the signs and symptoms of inflammation. Fortunately, there are anti-inflammatory agents like corticoids available commercially but are associated with side effect of immunity suppression. Anti inflammatory actions produced by certain herbs are found to be free from such side effects and are widely investigated presently to get promising results. Denaturation is the process in which proteins or nucleic acids lose their quaternary, tertiary and secondary structures by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), radiation or heat [1]. If proteins in a living cell are denatured, this results in disruption of cell activity and possibly cell death. Concentrated solutions of proteins in acid or alkali may become very viscous on denaturation [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are commercially available and are commonly used for treating chronic health problems like rheumatoid arthritis, osteoarthritis etc. Long term use of NSAIDS is also associated with side effects like stomach bleeding, allergic reactions, kidney problems, heart problems etc [8]. Thus, herbal medicines are maintaining their popularity not only for their historical and cultural use but also because of their safety with minimum or no side effects [2]. Plant material was procured in the month of November, 2016, from Gandhinagar, Mandsaur, India. Just after procurement, tea leaves were grounded mechanically into fine powder and kept into an air-tight container for use in the study. Indomethacin drug was procured commercially from market. All the other chemicals were of analytical grade obtained commercially. Double distilled water was used throughout the study. Powdered plant material (100 g) was extracted with distilled water/ethanol mixture (800 ml) by boiling under reflux for 45 min. The extracts were filtered and evaporated to dryness to yield the dry extracts of black tea (27.28%). The dry extract was kept in a vacuum desiccator until use. 25 mg, 75 mg and 125 mg of standard solution of indomethacin was dissolved in 100 ml of distilled water separately to get 250 µg/ml, 750 µg/ml and 1250 µg/ml solutions of indomethacin respectively. 25 mg, 75 mg and 125 mg of tea extract was dissolved in 100 ml of distilled water separately to get 250

µg/ml, 750 µg/ml and 1250 µg/ml of test solutions of extract respectively. The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of test solution so that final concentrations become 250, 750, 1250 µg/ml. Similar volume of double-distilled water served as control. Reaction mixtures were incubated at (37±2) ºC in a BOD incubator (Labline Technologies) for 15 min followed by heating at 70 ºC for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Indomethacin at the final concentration of (250, 750, 1250 µg/ml) was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 × (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration [3]. From the present investigation, it was found that the absorbance and percent inhibition of protein denaturation by hydroalcoholic tea extract and standard drug indomethacin increase with increase in their respective concentrations (250-1250 µg/ml). Although both tea extract and indomethacin were significantly efficient in reducing protein denaturation, standard drug indomethacin was found to be more effective. From the present study, it can be concluded that tea leaves possess marked in vitro anti-inflammatory effect against protein denaturation. Although the hydralcoholic extract of tea leaves was found to be less effective as compared to indomethacin drug, the present study provides useful information about the anti-inflammatory activity of tea leaves extract. Hydroalcoholic extract showed significant decrease in inflammation although the action was found to be less than that of standard drug. The present investigation will be supportive as additional information to the scientific evidences regarding *in vitro* studies. Since the correct mechanism of anti-inflammatory activity of the extract is not completely known till date, correlation between *in vitro* and *in vivo* investigations should be further studied to determine the phytochemical constituents responsible for the anti-inflammatory activity.

Figure 1: Reaction mixture containing varying concentrations of tea extract

Figure 2: Reaction mixture containing different concentrations of indomethacin

Table 1: Effect of tea extract on protein denaturation

S. No.	Concentration (µg/ml)	% Inhibition
1	Control	-
2	250	43.5
3	750	134
4	1250	220

Table 2: Effect of indomethacin on protein denaturation

S. No.	Concentration (µg/ml)	% Inhibition
1	Control	-
2	250	144
3	750	176
4	1250	258

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References

1. Sangita C, Priyanka C, Protapaditya D, Sanjib B. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed 2012: S178-S180.
2. Sridevi G, Sembulingam K, Muhammed I, Srividya S, Prema S. Evaluation of *in vitro* anti-inflammatory activity of *Pergularia daemia*. World J Pharm Res 2015: 4: 1100-1108.
3. Chatterjee P, Chandra S, Dey P and Bhattacharya. Evaluation of antiinflammatory effects of green tea and black tea: A comparative *in vitro* study. J Adv Pharm Technol Res 2012; 3: 136–138.

Acknowledgement

The author would like to pay their sincere thanks to colleagues and friends for their constant moral support and scientific advices during the whole work.

Conflict of Interest: None declared

Received: 24 November 2017, Revised: 12 December 2017, Accepted: 22 December 2017

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