Inflammation usually occurs when infectious microorganisms such as bacteria, viruses, or fungi invade the body, reside in particular tissues and/or circulate in the blood. At present, chronic inflammation is thought to be a risk factor for a broad range of age-related diseases such as hypertension, diabetes, atherosclerosis, and cancer. There are several possible factors that initiate and maintain a low-grade inflammatory response. These include aging, unbalanced diet, low level of sex hormones, and smoking. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost.

The rationale behind implementing this assay is that the denaturation of albumin protein leads to the formation of antigens which initiate type III hypersensitive reaction leading to inflammation. Nowadays, methotrexate (Mtx) is the most commonly prescribed drug in inflammatory disorders. Hence, Mtx was used as a reference standard drug to perform this assay.

Eucalyptus globulus is an evergreen tree native to Australia and a member of Myrtaceae family. Eucalyptus oil (EO) is an essential oil and having potent anti-inflammatory action Figure 1. The aromatic constituents of EO are used as analgesic, anti-inflammatory, and antipyretic remedies. EO contains a-pinene and 1,8-cineole and acts as an antioxidant, with strong radical scavenging activity.
et al. examined the role of eucalyptol as inhibitor of the production and synthesis of tumor necrosis factor-α, interleukin-1β, leukotriene B4, and thromboxane B2 in human blood monocytes, suggesting that eucalyptol is a strong inhibitor of cytokines that might be suitable for long-term treatment of airway inflammation in bronchial asthma and other steroid-sensitive disorders.\(^{14}\)

The Glycine max (G-max) is an annual legume of the Fabaceae family.\(^{16}\) It is indigenous to East Asia and China but now is extensively cultivated in many temperate regions of the world.\(^{17}\) Conventionally, soybean has been an excellent source of proteins\(^{18}\) [Figure 2]. Glyceollin on soybean can suppress inflammation by inhibiting the activation of nuclear factor-kappa (NF-kB), thus suppressing the increased production of pro-inflammatory cytokines.\(^{19}\) Glyceollin on soybean can suppress inflammation by inhibiting the activation of NF-kB, thus suppressing the increased production of pro-inflammatory cytokines.\(^{20}\)

Hence, turning to safe, effective, and time-tested ayurvedic herbal drug combination would be a preferable option and prime concerning the issue of our study.

**Materials and Methods**

**Plant material**

EO purchases from the local market. G-max is obtained from the soya bean seeds. Soya bean seeds purchase from the local market, then seeds are grind and then pass through sieve no. 85 and the powder of G-max were collected for the study.

**Chemicals**

Methotrexate (Imutrex) obtained from Cipla company. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

**Evaluation of in vitro anti-inflammatory activity**

**Protein denaturation using egg albumin\(^{21}\)**

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 aqueous extract of *Eucalyptus globulus* and G-max (50:50 ratio) so that final concentrations become 100, 200, 400, 800, and 1000 μg/ml. A similar volume of double-distilled water served as control. Then, the mixtures were incubated at 37 ± 2°C in a BOD incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. Methotrexate was used as a reference standard drug.

The percentage inhibition of protein denaturation was calculated using the following formula:

\[
\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs control}}.
\]

**Formula**

**Results and Discussion**

The effect of aqueous extract of *E. globulus* (EO) and G-max was evaluated against the denaturation of egg albumin and BSA. The results
are summarized in Figures 3 and 4. The combination of herbal extract exhibited significant anti-inflammatory activity at 100–1000 μg/ml by protein denaturation inhibition. The effect of the herbal extract was studied by comparing with the standard methotrexate.

The autoantigen production in inflammation is due to denaturation of protein and several studies reveal that protein denaturation is one of the reasons for inflammation.[24] Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well-documented causes of inflammation.[25] The decrease in absorbance of the test sample with respect to control indicated stabilization of protein, i.e., inhibition of protein (albumin) denaturation or anti-denaturation effect by the test extract and the reference drug methotrexate.[26]

The herbal extract at a concentration (1000 μg/ml) showed the highest percentage inhibition, i.e., 98.06% in egg and 97.28% in BSA in vitro assay. While methotrexate at concentration 100 μg/ml showed less effect compared to EO + G-max extract at a concentration (1000 μg/ml), i.e., 97.75% in egg and 93.01% in BSA in vitro assay, as shown in Figures 3 and 4. The maximum activity is exhibited by the herbal extract at a concentration of 1000 μg/ml. However, taking in consideration the side effects as well as costs of allopathic drugs, natural remedies from such herbs would be more preferable option for better results.

From the results of the present study, it can be stated that the combined extract of EO and G-max is capable of controlling the production of autoantigen and inhibiting the protein denaturation in inflammation study. Hence, E. globulus and G-max combination can be successfully used in the management of inflammation. The effect may be due to the synergistic effect rather than a single constituent.

**Conclusion**

The in vitro study by the inhibition of protein denaturation method conducted on the Ayurveda herbal plant combination concluded that the G-max and EO exhibited significant anti-inflammatory activity and hence can be used effectively in the management of inflammatory disorders.

**Acknowledgment**

I would like to express my special thanks of gratitude to the Management and Faculty of Department of Pharmacology, College of Pharmacy (Saswad), for providing all necessary facilities and encouragement.

**Authors’ Contributions**

P.S. wrote the first draft of the manuscript. P.J. first revised the manuscript. Other authors further improved the manuscript.

**References**

9. Franceschi C, Campiá J. Chronic inflammation (inflammaging) and its


