**INTRODUCTION**

Inflammatory diseases are major cause of morbidity and mortality in the world [1]. It is a body protective response against tissue injury and it involves a multifaceted array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and cell repair which are aimed at host defense mechanism activated in most ailment condition [2]. The critical role of inflammation as indicator many diseases that affect man, including autoimmune disorders, infection, cancer, cardiovascular diseases, inflammatory and neurodegenerative conditions. In the inflammatory process, it is important to understand the role of chemical mediators include vasoactive amines (histamine, serotonin), arachadonic acids (prostaglandins, leukotrienes) and cytokines (TNF and IL–1) come from plasma proteins or cells, including mast cells, platelets, neutrophils and monocytes/macrophages. Chemical mediators bind to specific receptors and can increase vascular permeability, neutrophil chemotaxis, stimulate smooth muscle contraction, induce pain or inflammation mediate oxidative damage. Most mediators are short-lived but produce harmful effects [3]. Anti-oxidants proceed as a major defense against free radical mediated toxicity, effective in the prevention and treatment of complex diseases like atherosclerosis, alzheimer disease, diabetes, cancer and stroke. Medicinal plants well known for millennia as a potential source of traditional therapeutic agents for the prevention of diseases and ailments [4]. Natural compounds like flavonoids and phenolic compounds are widely dispersed in medicinal plants which have been reported to large number of biological effects including antioxidants, anti-inflammatory and anticarcinogenic [5,6].

Lakshadi guggul is an ayurvedic polyherbal formulation, which is a combination of six Indian medicinal plants viz. Commiphora mukul, Cissus quadrangularis, Laccifer lacca, Sida veronicaefolia, Terminalia arjuna and Withania somnifera. It is traditionally, very useful in the management of inflammation. Current study investigated the in-vitro anti-inflammatory and antioxidant potential of successive extracts of a polyherbal formulation Lakshadi guggul tablet a combination of Indian medicinal plants viz. Commiphora mukul, Cissus quadrangularis, Laccifer lacca, Sida veronicaefolia, Terminalia arjuna and Withania somnifera. The studies were undertaken to assess the anti-inflammatory at doses levels of 200 mg and 400 mg/ml of each extract by in-vitro red blood cells membrane stabilization and antioxidant potential at doses levels of 125 μg, 250 μg and 500 μg/ml of each extract by DPPH free radical scavenging activity methods. In-vitro anti-inflammatory activity of extracts were found significant (P<0.01, *P<0.05) red blood cells membrane stabilization effects with 66.40 % in methanol, 62.15 % in ethyl acetate, 59.39 % in chloroform extract, and 66.18% with standard drug diclofenac. Similarly, in-vitro antioxidant activity of Lakshadi guggul tablets extracts were found significant reduction in free radical scavenging activity in 49.60% in methanol, 48.10% in ethyl acetate extract and relatively similar to 52.21% of standard drugs. The results of these studies revealed that Lakshadi guggul have strong potential antioxidant and anti-inflammatory agents.

**KEYWORDS:** Lakshadi guggul, in-vitro, anti-inflammatory, antioxidant, diclofenac etc
of low bone mineral density, osteoporosis, osteoarthritis and other diseases related to bones, teeth, joints, and muscles [7]. It has fascinated a great deal of research interest in natural herbal formulation i.e. Lakshadi guggul has been selected and scientifically validated the claim for antioxidants and anti-inflammatory activities. The present investigations scientifically validated the polyherbal formulation Lakshadi guggul for antioxidants and anti-inflammatory activities in vitro using DPPH free radical scavenging activity and human red blood cell membrane stabilization methods respectively.

**MATERIALS AND METHODS**

**In vitro Anti-inflammatory Activity**

**Red blood cell membrane stabilization method**

Human red blood cell membrane stabilization method performed as described by Kumar et al [3,8]. The blood sample collected from healthy volunteer who had not taken any medicines of NSAIDS family from 2 weeks prior to the experiment. Collected blood was mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3000xg rpm. The packed cells content washed with isosaline and 10% suspension was prepared for further use. Selected extracts of Lakshadi guggul tablets were assessed at doses levels of 125 μg and 500 μg/ml using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added in a test tube. These test tubes were incubated at 37°C for 30 min and centrifuged at 3000xg rpm for 20 min. The hemoglobin content of the supernatant solution was estimated with UV spectrophotometer at 560 nm. Diclofenac (50 mg/mL) was used as reference standard and a control was prepared by omitting the extracts. The experiment was performed in triplicate. The percentage of HRBC membrane stabilization or protection was calculated [10] by using the formula mentioned below:-

\[
\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} \times 100
\]

**In-vitro Antioxidant Activity - DPPH Free Radical Scavenging Assay Method**

Free radical scavenging activity of Lakshadi guggul extracts against stable 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams et al 1995 [9]. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in color (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol 6 × 10-5 M was prepared fresh each day before UV measurements. 3 ml of this solution was mixed with 125 μg, 250 μg and 500 μg/ml concentration of individual plant extract. The samples were kept in the dark place for 15 minutes at room temperature and the absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula.

\[
\text{Percentage Inhibition} = \frac{\text{(AB-AA)/AB}}{} \times 100
\]

Where AB = absorption of blank sample (t = 0 min)
AA = absorption of test extract solution

**RESULTS AND DISCUSSION**

**In-vitro Anti-inflammatory Activity**

In-vitro anti-inflammatory activity of Lakshadi guggul tablets were evaluated by using different extract like pet. ether, chloroform, ethyl acetate, methanol and aqueous extract by human red blood cell membrane stabilization method. The most significant in-vitro anti-inflammatory activity were found (P<0.01; 0.05) by red blood cells membrane stabilization effects with 66.40 % in methanol, 62.15 % in ethyl acetate, 59.39 % in chloroform extract, 50.18 % in aqueous extract and 66.18% with standard drug diclofenac. The results were tabulated in Table 1.

**In-vitro Antioxidant Activity**

In-vitro antioxidant activities of Lakshadi guggul tablets were evaluated for different extract like pet. ether, chloroform, ethyl acetate, methanol and aqueous extract at doses levels of 125 μg, 250 μg and 500 μg/ml by DPPH free radical scavenging activity methods. The results of Lakshadi guggul extract act as strong free radical scavengers as similar standard antioxidant i.e. Rutin used. Among all extracts, methanol extract of Lakshadi guggul tablets was shown maximum effect as antioxidant agent. The results were tabulated in Table 2.

**Table 1: HRBC membrane stabilization activity of Lakshadi guggul tablets**

| Groups            | Concentration (mg/kg) | IHP Activity (%Protection) |
|-------------------|-----------------------|-----------------------------|
| Control (vehicle only) | -                     | --                          |
| P.-E.L.G.         | 200                   | 40.18±0.61                  |
|                   | 400                   | 49.44±1.52                 |
| C.-E.L.G.         | 200                   | 54.31±1.27                  |
|                   | 400                   | 59.39±2.85                 |
| E.-E.L.G.         | 200                   | 44.08±1.34                 |
|                   | 400                   | 62.15±1.44                 |
| M.-E.L.G.         | 200                   | 62.59±1.20                 |
|                   | 400                   | 66.40±1.45                 |
| A.-E.L.G.         | 200                   | 49.58±1.791                |
|                   | 400                   | 50.18±1.30                 |
| Diclofenac        | 10                    | 66.18±1.82                 |

*E.-E.L.G.-Extract of Lakshadi Guggul, P.-E. P.- Pet. Ether, C.- Chloroform, E.- Ethyl acetate, M.- Methanol, A- aqueous, Diclofenac- standard drug used.Data observed in triplicate and statistically found significant as aP<0.01, bP<0.05
CONCLUSION

The in-vitro anti-inflammatory and antioxidant activities of successive extracts of Lakshadi guggul tablets may be due to the presence of active phytocconstituents such as flavonoids, coumarins, tannins and triterpenoids in different plants and their extracts and these may responsible for this activity. Therefore, Lakshadi guggul tablets can be used as a potent anti-inflammatory and antioxidant agent.

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Table 2: DPPH free radical scavenging activity of Lakshadi guggul tablets.

| Extracts of LG | Concentration (µg/ml) | Percentage of activity (±SE M) |
|---------------|-----------------------|-------------------------------|
| P-E.L.G.      | 125                   | 16.22±0.013                   |
|               | 250                   | 21.20±0.016                   |
|               | 500                   | 22.42±0.032                   |
| C-E.L.G.      | 125                   | 20.44±0.062                   |
|               | 250                   | 29.20±0.013                   |
|               | 500                   | 38.90±0.020                   |
| E-E.L.G.      | 125                   | 30.20±0.072                   |
|               | 250                   | 41.70±0.054                   |
|               | 500                   | 48.10±0.042                   |
| M-E.L.G.      | 125                   | 30.85±0.054                   |
|               | 250                   | 35.40±0.032                   |
|               | 500                   | 48.60±0.041                   |
| A-E.L.G.      | 125                   | 22.30±0.012                   |
|               | 250                   | 24.76±0.050                   |
|               | 500                   | 33.20±0.044                   |
| Rutin         | 125                   | 36.82±0.076                   |
|               | 250                   | 40.66±0.052                   |
|               | 500                   | 52.21±0.020                   |

*-E.L.G.-Extract of Lakshadi Guggul, P.E.- Pet. Ether, C.- Chloroform, E.- Ethyl acetate, M.- Methanol, A- Aqueous, Rutin- Standard drug used. Data observed in triplicate and statistically found significant as aP<0.01, bP<0.5