Journal of Medicinal Herbs and Ethnomedicine 2020, 6: 45-47
doi: 10.25081/jmhe.2020.v6.6238
http://updatepublishing.com/journal/index.php/jmhe

Short Communication

ISSN: 2455-0485

In-vitro anti-inflammatory and antioxidant potential of Triphala guggul tablets

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ABSTRACT

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 ($^{a}P<0.01$, $^{b}P<0.05$) 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

Received: April 20, 2020
Accepted: May 14, 2020
Published: May 18, 2020

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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 i.e. *Commiphora mukul*, *Cissus quadrangularis*, *Laccifer lacca*, *Sida veronicaefolia*, *Terminalia arjuna* and *Withania somnifera*. It is traditionally, very useful in the management

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 scientific 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 of 200 and 400 mg/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 37^0C for 30 min and centrifuged at 3 000xg 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:-

$$\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} \text{X } 100$$

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.

Percentage Inhibition = [(AB-AA)/AB] ×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 ($^aP<0.01$; $^bP<0.5$) 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[a]
C.-E.L.G.	200	54.31±1.27
	400	59.39±2.85[b]
E.-EL.G.	200	44.08±1.34[b]
	400	62.15±1.44[b]
M.-E.L.G.	200	62.59±1.20[b]
	400	66.40±1.45[a]
A.-E.L.G.	200	49.58±1.791
	400	50.18±1.30
Diclofenac	10	66.18±1.82[a]

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

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 [b]
E.-EL.G.	125	30.20±0.072
	250	41.70±0.054 [a]
	500	48.10±0.042 [a]
M.-E.L.G.	125	30.85±0.054
	250	35.40±0.032 [b]
	500	48.60±0.041 [a]
A.-E.L.G.	125	22.30±0.012
	250	24.76±0.050
	500	33.20±0.044 [b]
Rutin	125	36.82±0.076 [b]
	250	40.06±0.052 [a]
	500	52.21±0.020 [a]

*-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 [a]P<0.01, [b]P<0.5

CONCLUSION

The *in-vitro* anti-inflammatory and antioxidant activities of successive extracts of Lakshadi guggul tablets may be due to the presence of active phtoconstituents such as flavonoids, coumarins, tannins and tritrepenoids 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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