Research Article

Anti-Inflammatory Activity of Lavender Oil Using HRBC Membrane Stabilising Method

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ABSTRACT

The aim of study is to determine the anti-inflammatory effect of lavender oil using HRBC Membrane stabilising method *In-vitro.* Lavender extract was purchased and was analysed. Hypotonicity induced human red blood cell (HRBC) membrane stabilization method was performed to check the anti-inflammatory activity of lavender extract. Lavender extract was compared with a standard synthetic drug diclofenac to check the anti-inflammatory activity. Haemolysis and protective activity of both the drugs were checked and analysed. Results showed significant anti-inflammatory activity but was less compared to the standard drug diclofenac. Inflammation is the most common symptom for most of the diseases. Inflammation has to be treated prior to the disease treatment since decreasing pain is first step in a treatment procedure. This is where the Anti-inflammatory drugs act and eventually decrease the caused inflammation. Diclofenac being a synthetic drug can lead to a lot of side effects. Lavender oil being a natural drug has very less side effects comparatively and can be used in combination with other drugs in future in giving and efficient anti-inflammatory drug.

Keywords: lavender, anti-inflammatory, HRBC membrane stabilization, diclofenac.

INTRODUCTION

Lavandula which is commonly referred to as lavender is a genus of 39 known species of flowering plants from the family Lamiaceae.

It is native to the Mediterranean, Europe and Oceania Islands and were widely planted in United States such as in Yugoslavia and Hokkaido in Japan. The most widely cultivated species is Lavandula Angustifolia (or) English Lavender.[1,2] This plant is used as traditional medicine in different parts of the world for treatment of several disorders such a gastrointestinal, nervous and rheumatic disorders.[3]

Commercially, the plant is grown for the production of essential oil of lavender which can be used as:

- Antidepressant- used to alleviate depression.
- Analgesic- acting to relieve pain.
- Antiseptic- preventing the growth of disease-causing microorganisms
- Cicatrizant- promoting the healing of a wound or the formation of a cicatrix
- Expectorant- a medicine which promotes the secretion of sputum by the air passages, used to treat coughs.
- Nervine- used to calm the nerves.
- Vulnerary- use in the healing of wounds.

Apart from these medicinal uses, it can also be used as an ornamental plant and a pleasant fragrant. The major components of Lavandula Augustifolia Essential oil (LEO) are (-)-linalool and linayl acetate.[4]

Inflammation is a localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury. Inflammation is the most common reason for physician consultation in most developed countries. It is a major symptom in many medical conditions, and can interfere with a person's quality of life and general functioning.

Although a growing number of investigations have been conducted in these last years, there is a lack of more substantial data on the effects and mechanisms of action of lavender essential oil. In this work, Anti-inflammatory effect of Lavender Essential Oil and the effect was compared with standard anti-inflammatory synthetic drugs.

MATERIALS AND METHODS

The required materials were purchased from Cyprus.

The following method was used to analyse the anti-inflammatory effect[6].

Preparation of Blood Samples for Membrane Stabilization Assay

The human red blood cell (HRBC) membrane stabilization method has been used as a method to study the *in vitro* anti-inflammatory activity.

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl). All the blood samples were stored

at 4 °C for 24 h before use. It was centrifuged at 2500 rpm for 5 min and the supernatant was removed. The cell suspension was washed with sterile saline solution (0.9 % w/v NaCl) and centrifuged at 2500 rpm for 5 min. This was repeated three times till the supernatant was clear and colorless and the packed cell volume was measured. The cellular component was reconstituted to a 40 % suspension (v/v) with phosphate buffered saline (10 mm, pH 7.4) and was used in the assay.

Hypotonicity Induced Human Red Blood Cell (HRBC) Membrane Stabilization Method[6]

1.0 mL of test sample of different concentrations (20µg – 200 µg) in 1 ml of 0.2 M phosphate buffer and 0.5 mL of 10% HRBC suspension, 0.5 ml of 0.25 % hyposaline were incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared by distilled water instead of hypo saline to produce 100 % hemolysis without plant extracts. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following Formula:

$$\%\ of\ Hemolysis = \frac{Optical\ density\ of\ test\ sample}{Optical\ density\ of control} \times 100$$

$$\%\ Protection = 1 - \left[\frac{Optical\ density\ of\ test\ sample}{Optical\ density\ of\ control} \times 100\right]$$

RESULTSANDDISCUSSION

The following results were obtained in the performed experiments:

Haemolytic Activity

Table 1: Showing percentage haemolysis activities of Sample and Positive control (PC – Diclofenac)

	% Haemolysis	
Concentration (µg)	**Sample**	**PC**
20	43.29	23.95
60	39.11	20.04
100	37.92	18.71
120	33.86	16.82
160	29.33	13.57
200	24.72	9.89

The Lavender essential oil exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane.

The lysosomal membrane is similar to erythrocyte membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. The importance of stabilizing the lysosome membrane is in limiting the inflammatory response by preventing the release of lysomal constituents of activated neutrophil such as proteases and bactericidal enzymes which cause further tissue inflammation and damage upon extra cellular release.[7] Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The sample is compared with a positive control to check the percentage of heamolysis of the HRBC cells. When started with a minimal concentration of 20 (µg) the sample showed a haemolysis of 43.29%, while the positive control's activity on haemolysis was 23.95%.

As and when the concentrations were increased the haemolysis activity of both the sample and the positive control were decreased. When the concentration was 200 (µg), the sample's activity on heamolysis was 24.72% and the positive control's activity was 9.89%. So lesser the percentage of haemolytic activity, greater the anti-inflammatory activity. So in the obtained results, both the sample and the positive control showed anti-inflammatory effect, but the positive control being more efficient.

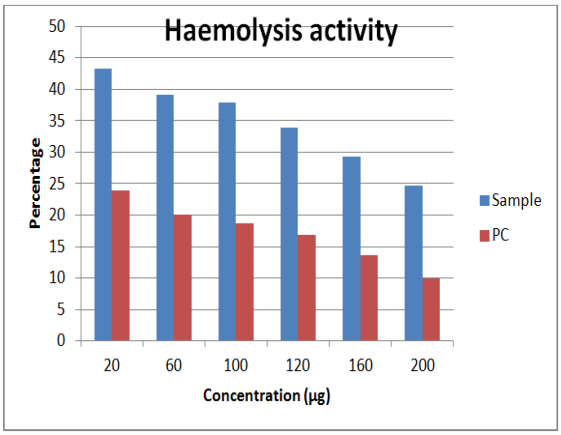

Figure 1: Showing percentage haemolysis activities of Sample and Positive control

PC – Diclofenac

Protective Activity

Table 2: Showing percentage protection activities of Sample and Positive control on HRBC

PC – Diclofenac

	% Protection	
Concentration (µg)	**Sample**	**Control**
20	56.71	76.05
60	60.89	79.96
100	62.08	81.29
120	66.14	83.18
160	70.67	86.43
200	75.28	90.11

The sample is compared with a positive control to check the percentage of protection of the HRBC cells. When

started with a minimal concentration of 20 (µg) the sample showed a protection value of 56.71%, while the positive control's activity on protection was 76.05%. As and when the concentrations were increased the protective ability of both the sample and the positive control were increased. When the concentration was 200 (µg), the sample's protective activity was 75.28% and the positive control's activity was 90.11%.

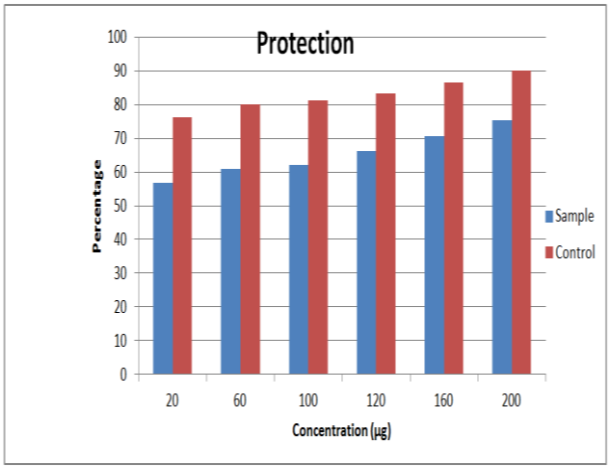

Figure 2: Showing percentage protection activities of Sample and Positive control on HRBC

PC - Diclofenac

CONCLUSION

Inflammation is the most common symptom for most of the diseases. Inflammation has to be treated prior to the disease treatment since decreasing pain is first step in a treatment procedure. This is where the Anti-inflammatory drugs act and eventually decrease the caused inflammation. Inflammation is the body's attempt at self-protection to remove harmful stimuli and begin the healing process.

Inflammation is part of the body's immune response. The first stage of inflammation is often called irritation, which then becomes inflammation - the immediate healing process.

Several *in vitro* and *in vivo* studies have reported the anti-inflammatory properties of lavender essential oil and its constituents. Lavender essential oil and constituents have been shown to interfere with key immunological pathways, e.g. nuclear factor kappa B (NF-κB) and p38 mitogen-activated protein kinase (MAPK) signalling as well as cytokine secretion.[8,9]

E.g., (+)-α-pinene, (-)-linalool and (+)-limonene were able to decrease interleukin-2 (IL-2) secretion and to increase the IL-10/IL-2 ratio in mouse primary splenocytes, which indicates their property to repress Th1 immune activation and suggest a potential inclination towards Th2.[8]

Furthermore, (-)-linalool was able to attenuate the production of lipopolysaccharide (LPS)-induced tumour necrosis factor α (TNFα) and IL-6 both in RAW 264.7 macrophages and in mice, and has been discussed as potential anti-inflammatory agent for preventing lung injury.[8,9]

In the above results obtained from the performed experiments, the positive control or standard (Diclofenac) has more efficient anti-inflammatory effect compared to lavender oil. But the standard being a synthetic drug, is associated with many side effects such as Abdominal or stomach bloating, burning, cramping, or pain, belching (bloody or black), tarry stools, cloudy urine, constipation, decrease in urine output or decrease in urine-concentrating ability, diarrhoea, dizziness, feeling of indigestion, headache, increased bleeding time, itching skin or rash, loss of appetite, nausea and vomiting, pain in the chest below the breastbone, pale skin, severe stomach pain, swelling, troubled breathing with exertion, unusual bleeding or bruising, unusual tiredness or weakness, vomiting of blood or material that looks like coffee grounds, weight loss.[10]

Comparatively Lavender extract being a natural compound has very less side effects and can be used in combination with other compounds for better anti-inflammatory activity in the future.

REFERENCES

1) "World Checklist of Selected Plant Families: Royal Botanic Gardens, Kew". kew.org.

2) Upson T, Andrews S. The Genus Lavandula. Royal Botanic Gardens, Kew 2004. ISBN 9780881926422. Retrieved 2012-03-30.

3) Silva, Gabriela L. DA. Antioxidant, analgesic and anti-inflammatory effects of lavender essential oil. An. Acad. Bras. Ciênc. [online]. vol.87, n.2, suppl. [cited 2016-05-07], 2015, 1397-1408.

4) Gostner JM, Ganzera M, Becker K. Lavender oil suppresses indoleamine 2,3-dioxygenase activity in human PBMC. BMC Complementary and Alternative Medicine. 14, 2014, 503. doi:10.1186/1472-6882-14-503.

5) ADAMS RP. Identification of essential oils by gas chromatography quadrupole mass spectrometry. JASMS, 16, 2001, 1902-1903. [Cross Ref]

6) Gandhisan R, Thamaraichelvan A, Baburaj. Anti-inflammatory action of Lannea coromandelica HRBC membrane stabilization. Fitotherapia, 62, 1991, 82-83.

7) Parvin MS, Das N, Jahan N, Akhter MA, Nahar L, Islam ME. Evaluation of *in vitro* anti-inflammatory and antibacterial potential of Crescentia cujete leaves and stem bark. BMC Research Notes. 8, 2015, 412. doi:10.1186/s13104-015-1384-5.

8) Ku CM, Lin JY. Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food Chem. 141, 2013, 1104–1113. doi: 10.1016/j.foodchem. 2013.04.044. [PubMed] [Cross Ref]

9) Huang MY, Liao MH, Wang YK, Huang YS, Wen HC. Effect of lavender essential oil on LPS-stimulated inflammation. Am J Chin Med. 40, 2012, 845–859. doi: 10.1142/S0192415X12500632. [PubMed][Cross Ref]

10) http://www.drugs.com/sfx/diclofenac-side-effects.html

11) Gandhisan R, Thamaraichelvan A. Baburaj: Anti-inflammatory action of Lannea coromandelica HRBC membrane stabilization. Fitotherapia. 62, 1991, 82–83. [Cross Ref]

12) Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf VO. Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil.Fitoterapia. 70, 1999, 251–257. doi: 10.1016/S0367-326X(99)00030-1. [Cross Ref]

13) Bauer AW, Kirby MM, Sherries JC, Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. Am J Clin Pathol. 45, 1966, 493–496. [PubMed]

14) Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S. *In-vitro* anti-inflammatory activity of leaf extracts of Basella alba linn. Var. alba. Int J Drug Dev Res. 3, 2011, 124–127.

15) Yurugasan N, Vember S, Damodharan C. Studies on erythrocyte membrane IV: *In vitro* haemolytic activity of Oleander extract. Toxicol Lett. 8, 1981, 33–38. doi: 10.1016/0378-4274(81)90134-X. [PubMed][Cross Ref]

16) Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti-inflammatory activity of leaves of Symplocos cochinchinensis (Lour) Moore ssp Laurina. Bangladesh J Pharmacol. 3, 2008, 121–124. doi: 10.3329/bjp.v3i2.956. [Cross Ref]

17) Yang GM, Wang D, Tang W, Chen X, Fan LQ, Zhang FF. Anti-inflammatory and antioxidant activities of Oxytropis falcate fractions and its possible anti-inflammatory mechanism. Chin J Nat Med. 8, 2010, 285–292. doi: 10.3724/SP.J.1009.2010.00285. [Cross Ref]

18) Sudharshan SJ, Prashith KTR, Sujatha ML. Anti-inflammatory activity of Curcuma aromatica Salisb and Coscinium fenestratum Colebr: a comparative study. J Pharm Res. 3, 2010, s24–25. [Cross Ref]

19) Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 4, 1999. [Cross Ref]

20) Hajhashemi V, Sajjadi SE, Heshmati M. Anti-inflammatory and analgesic properties of Heracleum persicum essential oil and hydroalcoholic extract in animal models. J Ethnopharmacol. 124, 2009, 475–480. doi: 10.1016/j.jep.2009.05.012. [PubMed] [Cross Ref]

21) Perry N, Perry E. Aromatherapy in the management of psychiatric disorders: clinical and neuropharmacological perspectives. CNS Drugs. 20, 2006, 257–280. doi: 10.2165/00023210-200620040-00001. [PubMed] [Cross Ref]

22) Mashhadi NS, Ghiasvand R, Askari G, Hariri M, Darvishi L, Mofid MR. Anti-Oxidative and Anti-Inflammatory Effects of Ginger in Health and Physical Activity: Review of Current Evidence. International Journal of Preventive Medicine. 4(Suppl 1), 2013, S36-S42. [Cross Ref]

23) Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J Ethnopharmacol. 127, 2010, 515–20. [PubMed] [Cross Ref]

24) Habib SH, Makpol S, Abdul Hamid NA, Das S, Ngah WZ, Yusof YA. Ginger extract (Zingiber officinale) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. Clinics (Sao Paulo). 63, 2008, 807–13. [PMC free article] [PubMed] [Cross Ref]

25) Dinarello CA. Anti-inflammatory Agents: Present and Future. Cell. 140(6), 2010, 935-950. doi:10.1016/j.cell.2010.02.043. [Cross Ref]

26) Wang Y, Wang VM, Chan C-C. The role of anti-inflammatory agents in age-related macular degeneration (AMD) treatment. Eye. 25(2), 2011, 127-139. doi:10.1038/eye.2010.196. [Cross Ref]

27) Elsayed EA, El Enshasy H, Wadaan MAM, Aziz R. Mushrooms: A Potential Natural Source of Anti-Inflammatory Compounds for Medical Applications. Mediators of Inflammation. 2014, 2014, 805841. doi:10.1155/2014/805841. [Cross Ref]

28) Beg S, Swain S, Hasan H, Barkat MA, Hussain MS. Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. Pharmacognosy Reviews. 5(10), 2011, 120-137. doi:10.4103/0973-7847.91102. [Cross Ref]

29) Vogl S, Picker P, Mihaly-Bison J. Ethnopharmacological in vitro studies on Austria's folk medicine—An unexplored lore *in vitro* anti-inflammatory activities of 71 Austrian traditional herbal drugs. Journal of Ethnopharmacology. 149(3), 2013, 750-771. doi:10.1016/j.jep.2013.06.007. [Cross Ref]

30) Keller WR, Kum LM, Wehring HJ, Koola MM, Buchanan RW, Kelly DL. A Review of Anti-Inflammatory Agents for Symptoms of Schizophrenia. Journal of psychopharmacology (Oxford, England). 27(4), 2013, 337-342. doi:10.1177/0269881112467089. [Cross Ref]

31) Vančo J, Gáliková J, Hošek J, Dvořák Z, Paráková L, Trávníček Z. Gold(I) Complexes of 9-Deazahypoxanthine as Selective Antitumor and Anti-Inflammatory Agents. Baptista PV, ed. PLoS ONE. 9(10), 2014, e109901. doi:10.1371/journal.pone.0109901. [Cross Ref]

32) Zhang Y, Zhao C, He W. Discovery and evaluation of asymmetrical monocarbonyl analogs of curcumin as anti-inflammatory agents. Drug Design, Development and Therapy. 8, 2014, 373-382. doi:10.2147/DDDT.S58168. [Cross Ref]

33) Kumar T, Jain V. Antinociceptive and Anti-Inflammatory Activities of Bridelia retusa Methanolic Fruit Extract in Experimental Animals. The Scientific World Journal. 2014, 2014, 890151. doi:10.1155/2014/890151. [Cross Ref]

34) Steel HC, Theron AJ, Cockeran R, Anderson R, Feldman C. Pathogen-and Host-Directed Anti-Inflammatory Activities of Macrolide Antibiotics. Mediators of Inflammation. 2012, 2012, 584262. doi:10.1155/2012/584262. [Cross Ref]

35) De Morais Lima GR, de Albuquerque Montenegro C, de Almeida CLF, de Athayde-Filho PF, Barbosa-Filho JM, Batista LM. Database Survey of Anti-Inflammatory Plants in South America: A Review. International Journal of Molecular Sciences. 12(4), 2011, 2692-2749. doi:10.3390/ijms12042692. [Cross Ref]

36) Li J, Du J, Liu D. Ginsenoside Rh1 potentiates dexamethasone's anti-inflammatory effects for chronic inflammatory disease by reversing dexamethasone-induced

resistance. Arthritis Research & Therapy. 16(3), 2014, R106. doi:10.1186/ar4556. [Cross Ref]

37) González Y, Torres-Mendoza D, Jones GE, Fernandez PL. Marine Diterpenoids as Potential Anti-Inflammatory Agents. Mediators of Inflammation. 2015, 2015, 263543. doi:10.1155/2015/263543. [Cross Ref]

38) Boukhatem MN, Kameli A, Ferhat MA, Saidi F, Mekarnia M. Rose geranium essential oil as a source of new and safe anti-inflammatory drugs. The Libyan Journal of Medicine. 8, 2013, 10.3402/ljm.v8i0.22520. doi:10.3402/ljm.v8i0.22520. [Cross Ref]

39) Chmiel JF, Konstan MW, Elborn JS. Antibiotic and Anti-Inflammatory Therapies for Cystic Fibrosis. Cold Spring Harbor Perspectives in Medicine. 3(10), 2013, a009779. doi:10.1101/cshperspect.a009779. [Cross Ref]

40) Kajal A, Bala S, Sharma N, Kamboj S, Saini V. Therapeutic Potential of Hydrazones as Anti-Inflammatory Agents. International Journal of Medicinal Chemistry. 2014, 2014, 761030. doi:10.1155/2014/761030. [Cross Ref]

41) Afsar T, Khan MR, Razak S, Ullah S, Mirza B. Antipyretic, anti-inflammatory and analgesic activity of Acacia hydaspica R. Parker and its phytochemical analysis. BMC Complementary and Alternative Medicine. 15, 2015, 136. doi:10.1186/s12906-015-0658-8. [Cross Ref]

42) Zhang Y, Liang D, Dong L. Anti-inflammatory effects of novel curcumin analogs in experimental acute lung injury. Respiratory Research. 16(1), 2015, 43. doi:10.1186/s12931-015-0199-1. [Cross Ref]

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