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Phytochemical Screening and Invitro Anti-Inflammatory Activity of Ethanolic Extract of *Centella asiatica*

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ABSTRACT

In ayurvedic medicine *Centella asiatica* is a valuable medicinal herbaceous creeper which has been valued for hundreds of years. Phytochemical analysis of *Centella asiatica*(Apiaceae) plant extracts revealed the presence of varied biochemical compounds like alkaloids, flavonoids, glycosides, phenolic compounds, triterpenoids and saponin etc. Since phenolic compounds, triterpenoids and flavonoids have remarkable anti-inflammatory, anti-arthritic and antioxidant activities, so our present work aims at evaluating the in vitro anti-inflammatory activity by Human Red Blood Cell (HRBC) membrane stabilization. To measure the anti-inflammatory activity, the inhibition of hypotonicity induced HRBC membrane lysis was used. The percentage Haemolysis was experimented from concentration of 50µg/ml to 2000µg/ml and the values reduced from 32.25% to 5.02%, on the other hand percentage Stabilisation in the same concentration range increased from 67.74% to 94.97%. Diclofenac sodium was used as the standard drug and the same experiment conducted in the same concentration range and the values of percentage haemolysis reduced from 47.18% to 1.24% and the percentage stabilisation increased from 52.81% to 98.76%. The results show that the extracts of *Centella asiatica* exhibited anti-inflammatory activities. *Centella asiatica* may be a profusely branched prostate herb consisting of active principles like Vallarine, Asiaticoside, Sitosterol, Tannins, Oxy asiaticoside. Asiaticoside is used in the treatment of leprosy. Sitosterol and tannin possess antiprotozoal and spasmolytic property. According to Siddha literature, the leaves *of Centella asiatica* are used for the treatment of syphilis, elephantiasis, all kinds of fever, abdominal disorder of children and hydrocele and these features are highlighted in this article.

Key words:
Centella asiatica,
Anti-inflammatory,
HRBC membrane stabilization.

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INTRODUCTION

In human body numerous physiological and biochemical processes may result in formation of different by-products such as oxygen centred free radicals and other reactive oxygen species. Overproduction of such by-products may result in oxidative damage to biomolecules (e.g. lipids, proteins, DNA),eventually resulting in many chronic diseases like atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans[1]. Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radicals scavenging molecules, such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines), Vitamins, terpenoids (including carotenoids), and endogenous metabolites, which are rich in antioxidant activity[2]. The intake of natural antioxidant has been associated with reduced risk of cancer, cardiovascular disease, diabetes and other diseases associated with aging. Inflammation is that the reaction of living tissues to injury, infection or irritation[3]. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid per oxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc[4]. Relating to acute or chronic inflammation, the extra cellular activity of these enzymes can be said.To measure invitro anti-inflammatory activity of the drugs or plant extracts the Stabilization of Human Red Blood Cell membrane (HRBC) by hypotonicity induced membrane lysis is used. Traditionally, *Centella asiatica* has been valued for hundreds of years in ayurvedic medicine for the treatment of leprosy, ulcer, asthma, bronchitis, elephantiasis, eczemas, anxiety, urethritis, cataract, eye troubles, diarrhoea among children, skin diseases, wound healing and for revitalizing the nerves and brain bells, hence primarily known as a "Brain food" or "Memory enhancer" in India. Biochemical compounds like alkaloids, flavonoids, glycosides, triterpenoids, saponins, amino acids, inorganic acids, vitamins, sterols and lipid compounds are found out during phytochemical analysis of extract of *Centella asiatica*[5].

MATERIAL AND METHODS

Preparation of Plant Extract

The entire plant material was collected in the month of August. Just after collection the plant material was washed thoroughly with running tap water and shade dried at room temperature(22-26^{0}c) and ground mechanically into a coarse powder.

By using petroleum ether, the powdered plant material was first defatted. The defatted plant material (45 gm) was extracted with 50% aqueous ethanol (400 ml)by boiling under reflux for 90 minutes. The extract was filtered and the solvent was separated by distillation and the

concentrated extract was evaporated to dryness to yield the dry extract. The dry extract was kept in a cool place.

Invitro anti-inflammatory bioassay

Preparation of Human Red Blood Cells (HRBC) Suspension: Fresh blood from human was collected and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water).[1]Then the blood was centrifuged for 10 min at 3000 rpm and packed cells were washed three times with isosaline (0.85%, pH 7.2).The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline[2].

Heat Induced Hemolysis

The principle involved is the stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer (pH 7.4, 0.15M), 2ml hyposaline (0.36%), 0.5ml HRBC suspension (10% v/v) with 0.5ml of plant extracts and standard drug diclofenac sodium of various concentrations (50,100,250,500,1000,2000 µg/ml) and control (distilled water instead of hyposaline to produce 100% hemolysis) were incubated at 37℃ for 30 min and centrifuged respectively[3]. Using spectrophotometer at 560nm the haemoglobin content in the suspension was estimated.

The percentage of HRBC membrane hemolysis is calculated as follows:

% Hemolysis=(Optical density of test sample/Optical density of control)*100

The percentage of HRBC membrane stabilization is calculated as follows: %Protection=100-[(Optical density of test sample/Optical density of control)*100]

CHEMICAL CONSTITUENTS SCREENING

The extract obtained was subjected to qualitative tests for the identification of various phytochemical constituents.

RESULT

Data Showing The Preliminary Phytochemical Screening of The Ethanolic Extract of *Centella asiatica*

(+) indicate positive test result

(-) indicate negative test result

Tab 1: Preliminary Phytochemical Screening of The Ethanolic Extract of *Centella asiatica*

TEST	RESULT
Test for carbohydrate	+
Test for proteins and amino acids	-
Test for glycosides	+
Test for flavonoids	+
Test for saponins	+
Test for coumarins	+
Test for tannins	+
Test for vitamins	+

The stabilization of HRBC membrane was taken as a measure of the anti-inflammatory activity. It is the inhibition of hypotonicity induced HRBC membrane lysis. At concentrations 50,100,250,500,1000,2000 µg/ml ,the percentage of membrane stabilization for ethanolic extract and diclofenac sodium were done. at different concentrations (50-2000 µg/ml) Ethanolic extracts of *Centella asiatica* are effective in inhibiting the heat induced hemolysis of HRBC. With the increasing concentration the membrane hemolysis is decreased and membrane stabilization/protection is increased. Anti-inflammatory activity of the extracts was concentration dependent.

Tab 2: Effect of *Centella asiatica*and standard on HRBC membrane hemolysis and membrane stabilization.

CONCENTRATION (µg/ml)	% HAEMOLYSIS OF *C.asiatica*	% STABILIZATION OF *C.asiatica*	% HEMOLYSIS OF Diclofenac sodium	% STABILIZATION OF Diclofenac sodium
20	32.25	67.74	47.18	52.81
100	20.77	79.22	12.47	76.54
250	16.05	84.05	18.68	81.32
500	12.43	87.56	14.34	85.67
1000	8.45	91.54	7.43	92.58
2000	5.02	94.97	1.24	98.76

DISCUSSION

There are certain problems associated with use of animals in experimental pharmacological research such as ethical issue and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for invitro assessment of anti-inflammatory and arthritic disease. Production of auto antigens in arthritic disease can be due to denaturation of tissue proteins. Agents that can prevent protein denaturation. Therefore, would be worthwhile for anti-inflammatory drug development.

CONCLUSION

Stabilization of the HRBCs membrane by hypotonicity induced membrane lysis was studied to determine the mechanism of anti-inflammatory action of *Centella asiatica.* Therefore, our invitro studies on *C.asiatica* extracts demonstrate Depression of inflammation. Hence, *Centella asiatica* are often used as a potent anti-inflammatory agent.

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REFERENCE

1. Kumbhare MR, Sivakumar T, Lakhote T, Morankar PG. An evaluation of membrane stabilizing activity and antimicrobial activity of stem bark of Moringa oleifera (Moringaceae) against selected microbes. American Journal of Drug Discovery and Development. 2014;4:41-9.
2. Chippada SC, Volluri SS, Bammidi SR, Vangalapati M. In vitro anti-inflammatory activity of methanolic extract of *Centella asiatica* by HRBC membrane stabilisation. Rasayan J Chem. 2011;4(2):457-60.

3. Parameswari P, Devika R, Vijayaraghavan P. In vitro anti-inflammatory and antimicrobial potential of leaf extract from Artemisia nilagirica (Clarke) Pamp. Saudi journal of biological sciences. 2019 Mar 1;26(3):460-3.
4. Sivakrishnan S, Veeramani G. Phytochemical Activity Of Aerial Parts Of Cordia ObliquaWilld. International Research Journal of Pharmacy. 2019Feb;10(7):94–8.
5. Mamtha B, Kavitha K, Srinivasan KK, Shivananda PG. An in vitro study of the effect of *Centella asiatica* [Indian pennywort] on enteric pathogens. Indian Journal of Pharmacology. 2004 Jan 1;36(1):41.
6. Antony B, Santhakumari G, Merina B, Sheeba V, Mukkadan J. Hepatoprotective effect of *Centella asiatica* (L) in carbon tetrachloride-induced liver injury in rats. Indian Journal of Pharmaceutical Sciences. 2006;68(6):772.
7. Sarumathi A, Saravanan N. A study on the hematological parameters and brain acetylcholine esterase activity in immobilization induced stress and co-treatment with *Centella asiatica* leaves extract to wistar rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2013 Apr 1;3(2):102.
8. Meena H, Pandey HK, Pandey P, Arya MC, Ahmed Z. Evaluation of antioxidant activity of two important memory enhancing medicinal plants Baccopamonnieri and *Centella asiatica*. Indian journal of pharmacology. 2012 Jan;44(1):114.
9. Raghavendra M, Maiti R, Kumar S, Trigunayat A, Mitra S, Acharya SB. Role of *Centella asiatica* on cerebral post-ischemic reperfusion and long-term hypoperfusion in rats. International Journal of Green Pharmacy (IJGP). 2009;3(2).
10. Dr Kokate C.K; Purohit A.P; Ghokhale S.B; Introduction in : Text bookof Pharmacognosy, NiraliPrakashan, Vol.18,2002.
11. Ayurvedic Encyclopedia- 3.
12. Tripathi K..D Essentials of Medical pharmacology. 6th ed JaypeeBrothers Medical publishers(P)Ltd.: New Delhi; 2008.
13. Ghani A. Medicinal plants of Bangladesh. The Asiatic Society ofBangladesh : Dhaka:2003.
14. Natural Products, RaphelIkan , 2nd EditionComprehensive practical organic chemistry, V.k. Ahluwalia, RenuAggarwal.
15. V. Rajendran, K.S. Lakshmi., Bangladesh J Pharmacol., year 2008, Vol3, Page No 121-124.
16. M. N. A. Sreejayan Rao., J Pharm Pharmacol., Year 1997, Vol 49,Page No 105-107.
17. Kusanop .G. Orihara.S et al . , Chem.Pharm Bull,2002 Vol 50, 177-192
18. K. K. Kakkar., Indian Drugs., Year 1988, Vol 26, Page no 92-97.
19. Martindale , The extra Pharmacopeia.
20. Indian Medicinal Plants - Author: Orient Longmann Page No.52.
21. Handbook of Medicinal Plants - Author: Dr. P.N.V. Kurup Page No.61.
22. Medicinal Plants Authors: Robert Bentley and Henry Trimen Page No:117.
23. Medicinal Plants and Raw Drugs of India Authors: Purshotam Kaushik & Anil kumardhiman.