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Evaluation of *in vitro* and *invivo* anti-inflammatory activities of *Parthenium camphora*

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Abstract

The present investigation was carried out to evaluate the anti-inflammatory potential of solvent extracts of *Parthenium camphora* (Family: Compositae), a non-useful and waste weed growing through waste sides. The anti-inflammatory activities were assessed through *in vitro* and *in vivo* procedures, the results were found to be very surprising and promising. Aqueous and Ethanolic solvent extracts of *Parthenium camphora* were found to have significant anti-inflammatory activity at doses 100 and 120 mg/Kg during *in vitro* anti-inflammatory assay. The ethanolic fractions of the plant causes significant reduction in inflammation i.e. 92 % (120 mg/kg) followed by aqueous extract i.e. 85 % (120 mg/kg) compared to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.10 ± 0.05, 0.14 ± 0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration. Ethanolic extracts showed potent anti-inflammatory activity in comparison to aqueous extracts. The extracts showed higher anti-inflammatory potential as the dose varies. Thus results showed that extracts showed significant anti-inflammatory activity in dose-dependent manner. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bacterial enzymes and proteases which cause further tissue inflammation and damage. From the above study it was concluded that the ethanolic extract of *Parthenium camphora* has significant membrane stabilization property compared to the aqueous extract of the same plant and it was comparable to the standard drug Diclofenac Sodium.

Keywords: Anti-inflammatory activities, *Parthenium camphora*, aqueous and ethanolic solvent extracts, carrageenan induced animal model, membrane stabilization

INTRODUCTION

Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto medicine for the treatment of disease [1]. It is estimated that plant materials are present in, or have provided the models for 50% Western drugs [2]. The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments [3]. Many plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today, the search for natural compounds rich in antimicrobial, antioxidant and anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic disorders (cancer, diabetes, arthritis, hypertension etc). Ant-inflammatory activities and active principles from plants of North West Himalaya Garhwal region were determined [4-8]. Natural products derived from plants offer a new source of biological that may have a great impact overall human health [9]. Typical inflammatory diseases such as rheumatoid arthiritis, asthma, colitis and hepatitis are among the causes of death and disability in the world [10-12]. Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti inflammatory action [13]. Inflammatory response is a series of well coordinated dynamic mechanism consisting of specific vascular, humoral and cellular events that is characterized by the movement of fluids, plasma and inflammatory leukocytes (neutrophils, eosinophils, basophils and macrophages) to the site of inflammation [14, 15]. A variety of chemical mediators or signaling molecules such as histamine, serotonin, leukotrienes, prostaglandins and oxygen derived free radicals (O2, OH, $ONOO^-$) are produced by inflammatory and phagocytic cells predominantly in the sequences which participates in onset of inflammation [16, 17]. In the present investigation we have reported the *in vitro* and *in vivo* anti-

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inflammatory activities of different solvent extracts of whole plant of *P. camphora.*

MATERIALS AND METHODS

The chemicals and reagents used were of Analytical Grade and were procured from Ranbaxy and CDH. The animal house used was ethical committee approved in HIHT University, Dehradun (U.K), India.

Collection of Plant material

The whole plant, *Parthenium camphora* belonging to Compositae family was selected for the study. The herbariums of plant material were prepared and were further identified by Dr. Ajai Swami, Chinmaya Degree College, Haridwar (U.K), India. Whole plant material of *Parthenium* was dried under shade and ground to form the fine powder.

Preparation of Solvent Extracts

The powdered plant material was soaked in approximately 400 ml of ethanol and water separately on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatmann filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50^{o}C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4^{0}C. The extracts doses were optimized by determination of LD50 of the dose. The dose at which death of the 50 % of the animal population in the group occurs is known as LD50.

In vitro studies for determination of anti-inflammatory potential

(A) The human red blood cell (HRBC) membrane stabilization method

The method as prescribed [18, 19] was adopted with some modifications 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) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared (100 and 120 mg/ml) using distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37^{0}C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

Percent Protection (%) = (100- OD of drug treated sample/OD of Control) X 100

(B) Inhibition of Albumen Denaturation

Method as prescribed [19] was followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Control-OD of Sample/OD of Control) X100

(C) Heat induced hemolysis

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10 % RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 minutes. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent hemolysis was calculated by the formula mentioned in the above procedure.

In vivo studies for determination of anti-inflammatory potential

Animals

Extracts of whole plant of *Parthenium camphora* were evaluated. Male albino rats (180–200 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals [20]. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1ºC with a 12 h light/dark cycle. The *in vivo* anti-inflammatory activity was performed in HIHT University, Dehradun (U.K), India. The institutional animal ethical committee has approved the protocol of the study.

Carrageenan-induced edema in rats

For screening *in vivo* anti-inflammatory activity for each of the extracts, 5 Groups of five animals each were used.

Group I: Treatment with Vehicle/Control (Distilled water); 10 ml/Kg
Group II: Treatment with Vehicle/Control (Ethanol); 10 ml/Kg
Group III: Treatment with Ethanolic extract of whole plant of *P. camphora* (Test); 100 & 120 mg/Kg
Group IV: Treatment with Aqueous extract of whole plant of *P. camphora* (Test); 100 & 120 mg/Kg
Group V: Treatment with Standard drug, Diclofenac Sodium (10 mg/Kg)

Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The solvent extracts of plant at dose of 100 and 120 mg/kg were administered orally 60 minutes before carrageenan injection. Diclofenac Sodium (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a

plethysmometer at time 0, 1, 2, 3, and 4 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

RESULTS

Aqueous and Ethanolic solvent extracts of *Parthenium camphora* were found to have significant anti-inflammatory activity at doses 100 and 120 mg/Kg during *in vitro* anti-inflammatory assay. During *in vivo* anti-inflammatory activity, the paw edema was reduced significantly in carrageenan induced albino rats through introduction of ethanolic extracts at a dosage 120 mg/Kg. Ethanolic extracts showed potent anti-inflammatory activity in comparison to aqueous extracts. The extracts showed higher anti-inflammatory potential as the dose varies. Thus results showed that extracts showed significant anti-inflammatory activity in dose-dependent manner.

Table 1. Percent activity of HRBC membrane stabilization

S.No.	Groups	Concentration of Extracts of *Parthenium camphora*	Percent Protection± SD
I	Control	100 mg/ml	-
		120 mg/ml	-
II	Ethanolic extract	100 mg/ml	56.2 ±0.06
		*120 mg/ml	72.5 ±0.06
III	Aqueous extract	100 mg/ml	46.5 ±0.06
		*120 mg/ml	68.2 ±0.06
IV	Standard (Diclofenac Sodium)	100 mg/ml	72.34 ±0.06
		*120 mg/ml	83.54 ±0.06

*Potent Extracts/Drug

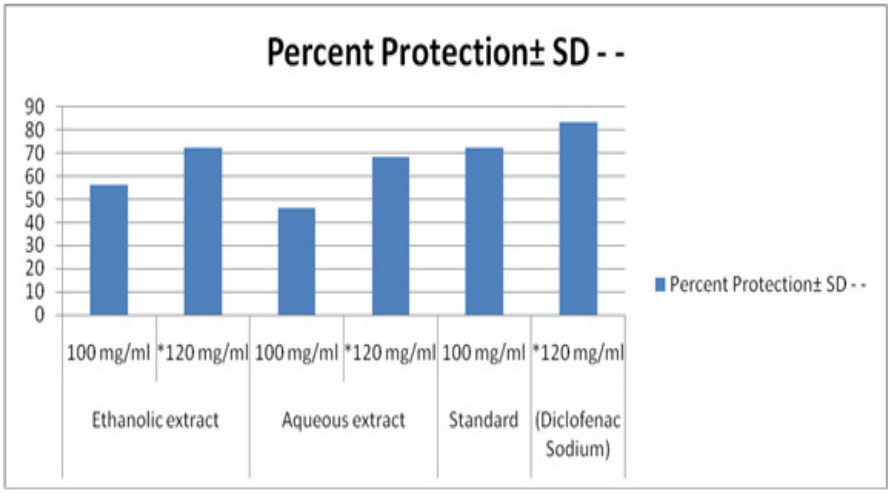

Fig 1. HRBC (Human Red Blood Cell) Membrane Stabilization Assay

Table 2. Percent Inhibition of Albumen Denaturation

S.No.	Groups	Concentration of Extracts of *Parthenium camphora*	Percent Protection± SD
I	Control	100 mg/ml	-
		120 mg/ml	-
II	Ethanolic extract	100 mg/ml	76.2 ±0.06
		*120 mg/ml	89.61±0.06
III	Aqueous extract	100 mg/ml	72.5 ±0.06
		*120 mg/ml	86.81±0.06
IV	Standard (Diclofenac Sodium)	100 mg/ml	78.54 ±0.06
		*120 mg/ml	93.54 ±0.06

*Potent Extracts/Drug

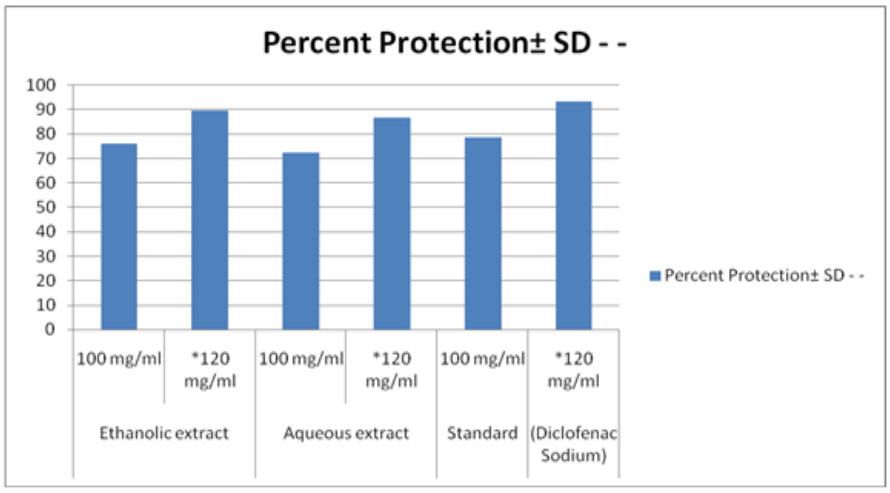

Fig 2. Percent Inhibition of Albumen Denaturation

Table 3. Percent Inhibition of Heat Induced Hemolysis

S.No.	Groups	Concentration of Extracts of *Parthenium camphora*	Percent Protection± SD
I	Control	100 mg/ml	-
		120 mg/ml	-
II	Ethanolic extract	100 mg/ml	56.2 ±0.06
		*120 mg/ml	78.82±0.06
III	Aqueous extract	100 mg/ml	42.5 ±0.06
		*120 mg/ml	76.65±0.05
IV	Standard (Diclofenac Sodium)	100 mg/ml	78.54 ±0.06
		*120 mg/ml	85.92 ±0.05

*Potent Extracts/Drug

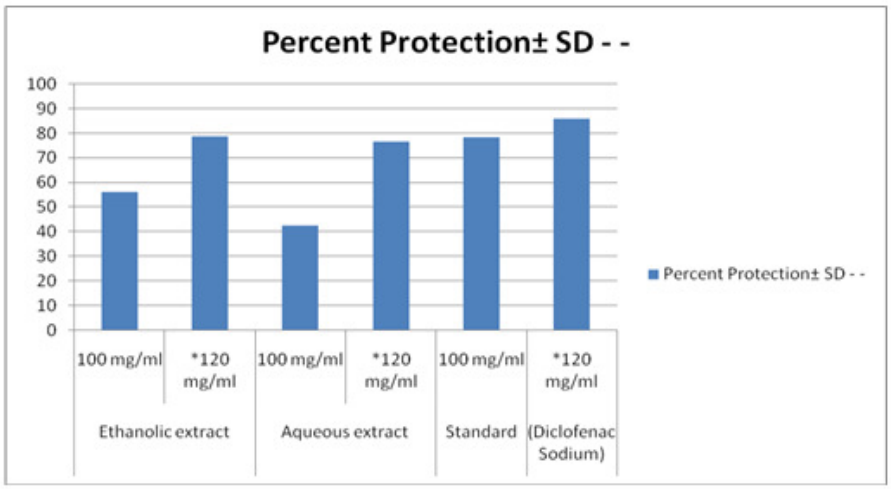

Fig 3. Percent Inhibition of Heat Induced Hemolysis

Carrageenan-induced edema in rats

Table 4: Anti-inflammatory activities of different extracts of *Parthenium camphora*

					Paw volume (ml) ± SD	
Experiment	**Control**	**Diclofenac Sodium (10 mg/kg orally)**	**Ethanol extract (120 mg/kg)**	**Aqueous extract (120 mg/kg)**	**Ethanol (120 ml/kg)**	**Distilled water (120 ml/kg)**
1h after treatment	0.25±0.05	0.21±0.05	0.23± 0.05	0.28±0.003	0.20±0.05	0.34±0.05
2h after treatment	0.25±0.05	0.18±0.05	0.20± 0.05	0.24±0.05	0.15±0.05	0.34±0.05
4h after treatment	0.25±0.05	0.16±0.05	0.10± 0.05	0.14±0.05	0.30±0.05	0.34±0.05

±, S.D, Standard Deviation

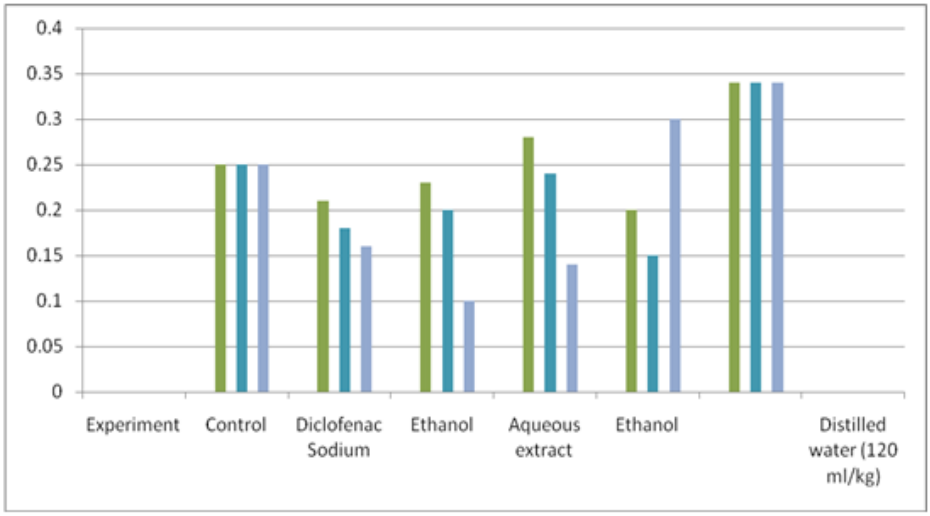

Fig 4. Reduction in paw volume (volume of water displaced) by treatment of different extracts of *Parthenium camphora* and treatment of positive and negative controls.

In vitro studies for determination of anti-inflammatory potential

(A) The human red blood cell (HRBC) membrane stabilization method:

Amongst aqueous and ethanolic extracts, the ethanolic extracts at a concentration of 120 mg/ml showed 72.5 ±0.06 % protection of HRBC in hypotonic solution in comparison to aqueous extracts (68.2 ±0.06 %). The results were compared with standard Diclofenac Sodium which showed 83.54 ±0.06 % protections at 120 mg/ml. The results are shown in Table 1 and Figure 1. *Parthenium camphora* whole plant extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [21] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [22]. Some of the NSAIDs are known to posses membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. 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 extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [23].

(B) Inhibition of Albumen Denaturation:

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. The ethanolic extracts were found to be effective in inhibiting heat induced albumin denaturation in comparison to aqueous extracts at a dose of 120 mg/ml. Maximum inhibition 89.61±0.06 was observed from ethanolic extracts followed by aqueous extracts (86.81±0.06). The results were compared with standard Diclofenac Sodium which showed 93.54 ±0.06 % inhibition in albumen denaturation at 120 mg/ml. The results are reported in Table 2 and Figure 2. Since during inflammation condition, protein of the cell gets denatured, thus here albumen protein is used as a model whose protection in denaturation by plant extracts was studied.

(C) Heat induced hemolysis:

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different water extract of *Parthenium camphora*. Both the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree. The maximum inhibition was recorded 78.82±0.06 % from ethanolic extract followed by aqueous extracts (76.65±0.05 %). The results were compared with standard Diclofenac Sodium which showed the maximum inhibition 85.92 ±0.05 % at 120 mg/ml. The results are reported in Table 3 and Figure 3. Heat induced hemolysis method is another method depicting HRBC membrane stabilization.

In vivo studies for determination of anti-inflammatory potential

Carrageenan-induced edema in rats

The anti-inflammatory effects of the solvent extracts of *Parthenium camphora* on carrageenan-induced edema in rat's hind paws are presented in Table 4 and Figure 4. The anti-inflammatory activities of extracts were found to have effect in dose-dependent manner. There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, ethanolic extract and aqueous extract (120 mg/kg) showed a significant reduction in the edema paw volume. There was no reduction in inflammation found in case of rats treated with ethanol and distilled water. The results showed that ethanolic fractions of the plant causes significant reduction in inflammation i.e. 92 % (120 mg/kg) followed by aqueous extract i.e. 85 % (120 mg/kg) compared to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.10 ± 0.05, 0.14 ±

0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration.

DISCUSSION

In the present investigation carried out to evaluate the anti-inflammatory potential of solvent extracts of *Parthenium camphora* (Family: Compositae) through *in vitro* and *in vivo* procedures, the results were found to be very surprising and promising. Parthenium is a waste plant and a weed growing randomly in fields and at the road sides. The pollen grains dispersal through air and other sources can leads to its uncontrolled growth. One cannot imagine its potential pharmacological properties. The previous studies reported its pharmacological properties and anti-inflammatory behavior [24-27]. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [21] and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage [22]. From the above study it was concluded that the ethanolic extract of *Parthenium camphora* has significant membrane stabilization property compared to the aqueous extract of the same plant and it was comparable to the standard drug Diclofenac Sodium. Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation. Similar results were observed from many reports from plant extract [19]. From the above study it was concluded that the ethanolic extract of *Parthenium camphora* had maximum albumen denaturation protection property as compared to the aqueous extract of the same plant and it was comparable to the standard drug Diclofenac Sodium. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The precise mechanism of this membrane stabilization is yet to be elucidated; it is possible that the *Parthenium camphora* produced this effect by reducing the surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins. The above findings also confirmed that ethanolic extracts of *Parthenium camphora* possessed maximum protection activity of RBC membrane by heat induction in comparison to aqueous extracts. This can be due to the reduction in heat induced by inflammation (associated with any disease) by the effect of plant extracts. When the extracts were evaluated for in vivo anti-inflammatory activity on carrageenan induced albino rats, the ethanolic and aqueous extracts also showed the similar pattern as that of in vitro studies. Amongst the extracts, ethanolic extracts at 120 mg/Kg showed maximum reduction in paw edema in comparison to aqueous extracts. The ethanolic fractions of the plant causes significant reduction in inflammation i.e. 92 % (120 mg/kg) followed by aqueous extract i.e. 85 % (120 mg/kg) compared to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.10 ± 0.05, 0.14 ± 0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration. The studies thus confirmed that Parthenium camphora is a potent anti-inflammatory agent. The findings of the study corelate the previous studies reported [24, 25, 27]. Further studies in future context on the basis of present studies are however needed to isolate the active principle (s) responsible for anti-inflammatory potential.

REFERENCES

[1] Iwu M.W., A.R. Duncan, C.O. Okunji. 1999. New antimalarials of plant origin. In: Janick J, Editor. Perspective on new crops and new uses. Alexandria: VA ASHS Press, pp. 457-462

[2] Rodders J., M. Speedie, V. Tyler. 1996. Pharmacognosy and Pharmacobiotecknology. Baltimore: Williams and Wilkins, pp. 1-14.

[3] Bandow J.E., H. Brotz, L.I.O. Leichert, H. Labischinski, M. Hecker. 2003. Proteomic approach to understanding antibiotic action. *Amicro. Agents. Chemother.,* 47: 948-955

[4] Mathur A., S.K. Verma, R. Purohit, S.K. Singh, D. Mathur, GBKS Prasad, V.K. Dua. 2010. Pharmacological investigation of *Bacopa monnieri* on the basis of antimicrobial, antioxidant and anti-inflammatory properties. *Journal of Chemical and Pharmaceutical Research.* 2(6): 191-198.

[5] Mathur A., R. Purohit, D. Mathur, GBKS Prasad, V.K. Dua. 2011. Pharmacological investigation of methanol extract of *Syzigum cuminii* seeds and *Crateva nurvula* bark on the basis of antimicrobial, antioxidant and anti-inflammatory properties. *Der Chemica Sinica.* 2(1): 174-181.

[6] Mathur A., V. Gupta, S.K. Verma, S.K. Singh, A. Prakash, GBKS Prasad, V.K. Dua. 2011. Anti-inflammatory activity of different fractions of *Leucas cephalotes* leaves extract. *International Journal of Current Pharmaceutical Review & Research.* 1(3): 28-32.

[7] Mathur A., S.K. Verma, S.K. Singh, D. Mathur, GBKS Prasad, V.K. Dua. 2011. Investigation of anti-inflammatory properties of *Swertia chirayta* and *Gloriosa superba*. *Recent Research in Science & Technology*. 3(3): 40-43.

[8] Mathur A., S.K. Verma, S.K. Singh, GBKS Prasad, V.K. Dua. 2011. Investigation of the antimicrobial, antioxidant and anti-inflammatory activity of the compound isolated from *Murraya koenigii*. *International Journal of Applied Biology and Pharmaceutical Technology*. 2(1).

[9] Baladrin M.F. J.A. Klocke, E.S. Wurtele. 1985. Natural plant chemicals: sources of industrial and medicinal plants. *Sci.,* 228: 1154-1160.

[10] Naik D.G, A.M. Majumdar, C.N. Dandge, H.M. Puntambekar. 2000. Anti-inflammatory activity of *Curcuma amada* Roxb. in Albino rats. *Indian J. Pharmacology*. 32: 375-377.

[11] Goudgaon N.M., N.R. Basavaraj, A. Vijayalaxmi. 2003. Anti-inflammatory activity of different fractions of *Leucas aspera* Spreng. *Indian J. Pharmacology*. 35: 397-398.

[12] Abreu P., S. Matthew, T. Gonzalez, D. Costa, M.A. Segundo, E. Fernandes. 2006. Anti-inflammatory and antioxidant activity of a medical tincture from *Pedilanthus tithymaloides*. *Life Sciences*. 78: 1578-1585

[13] Erdemoglu N., E.K. Akkol, E. Yesilada, I. Calis. 2008. Bioassay-guided isolation of anti-inflammatory and antinociceptive

principles from a folk remedy, Rhododendron ponticum L. leaves. *Journal of Ethnopharmacology*. 119(1): 172-178.

[14] Gokhalae A.B., A.S. Damre, K.R. Kulkarni, M.N. Saraf. 2002. Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S.lappa*, *A.speciosa* and *A.aspera*. *Phytomedicine.* 9:433-437.

[15] Hou C., T. Kirchner, M. Singer. 2004. In vivo activity of phospholipase C inhibitor in chronic and acute inflammatory reactions. *J. Pharmacology Exp. Therapeutics*. 309: 609-704.

[16] Vijyalakshmi T., V. Muthulakshmi, P. Sachdanandam. 1997. Salubrious effect of Semecarpus anacardium against lipid peroxidative changes in adjuvant arthritis studied in rats. *Mol. Cellular Biochem.* 175: 65-69.

[17] Safayhi H., E.R. Sailer. 1997. Anti-inflammatory actions of pentacyclic triterpenes. *Planta Med.,* 63: 487-493

[18] Gopalkrishnan S., M. Kamalutheen, T.S. Ismail. 2009. Anti-inflammatory and anti-arthritic activities of *Merremia tridentata*. *E-Journal of Chemistry*. 6(4): 943-948.

[19] Sakat S., A.R. Juvekar, M.N. Gambhire. 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *Int. J. Pharm. Pharmacol. Sci.* 2(1): 146-155.

[20] Olfert E.D., B.M. Cross, A.A. McWilliams. 1993. Canadian Council of Animal Care guide to the care and use of experimental animals, 2nd edition, Vol.1.

[21] Chou C.T., 1997. The anti-inflammatory effect of Tripterygium wilfordii Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. *Phytother Res.* 11,152-154.

[22] Murugasan N., S. Vember, C. Damodharan. 1981. Studies on erythrocyte membrane IV. In vitro haemolytic activity of Oleander extract. *Toxicol Lett*.8: 33-38.

[23] Vane J.R., J.A. Mitchell, I. Appleton, A. Tomlinson. 1994. Inducible isoforms of cyclo-oxygenase and nitric oxide synthase in inflammation. Proceedings of National Academy of Sciences., USA: 2046-2050

[24] Tiuman T.S., T.U. Nakamura, DAG. Cortez. 2005. Anti-leishmanial activity of Parthenolide, a Sesquiterpene lactone isolated from Tanacetum parthenium. *Antimicrobial agents and Chemotherapy*. 49(1): 176-182

[25] Bussa S.R., P. Bandela. 2010. Analgesic activity of Parthenium camphora in mice models of acute pain. *Int. J. Pharm. Res. Dev*. 2(6): 1-7.

[26] Rao P.V.S., N. Harindranath, T.R. Narasimhan. 1984. Characterization of a toxin from Parthenium hysterophorus and its mode of excretion in animals. 6(5): 729-738.

[27] Shafagat A. 2008. Extraction and Determining of Chemical structure of Flavonoids in Tanacetum parthenium (L.) Schultz Bip. from Iran. *JSIAU.* 18(68): 39-42.