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Research Article

Assessment of *In vitro* Anti-Inflammatory Activity of Ginger and Diclofenac Sodium Combination

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ABSTRACT

The present research work aimed at evaluating the anti-inflammatory activity of *Zingiber officinale* with diclofenac sodium by human red blood cell (HRBC) membrane stabilization and protein denaturation. The precluding of hypotonicity-induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. The percentage of membrane stabilization at different concentrations was performed for methanolic, hydro-methanolic ginger extract, and diclofenac sodium. At a dose of 50 µg/mL, the maximum membrane stabilization of 86.34% was found for ginger extract (test), and at a dose of 500 mcg/mL, membrane stabilization was found to be 91.16% for diclofenac sodium (standard), and the membrane stabilization for combination (ginger with diclofenac sodium) at a dose of 50 µg/mL was recorded at 86.43%, as the concentration increased (1,000 mcg/mL) for combination (ginger with diclofenac sodium), the percentage protection was decreased. *In vitro* protein denaturation was performed by using the egg albumin method. Maximum inhibition was observed in the case of methanolic extract of ginger at concentration 1,000 mcg/mL, and it was 78.83 ± 5.17, and in hydro-methanolic extract for diclofenac sodium at concentration 1,000 mcg/mL and it was 63.37 ± 2.78. Minimum inhibition was observed in combination with methanolic extract of ginger and diclofenac sodium at concentration 1,000 mcg/mL, and it was 25.27 ± 1.76, and in the combination of the hydro-methanolic extract of ginger and diclofenac sodium at concentration 1,000 mcg/mL and it was 28.23 ± 3.14. This study's results divulge that a low dose combination of ginger and diclofenac sodium has higher anti-inflammatory activity than diclofenac sodium and ginger alone. With this initial study, research work could be extended further; therefore, the particular pharmacological action for the combination of ginger with diclofenac sodium could be discovered.

Introduction

Inflammation is a physiological response that secures the body from tissue injury. Acute inflammation occurs very rapidly, and its main features are the release of fluid and various plasma proteins. The process of acute inflammation can last for a few or several minutes to several days. Chronic inflammation takes place when the acute inflammatory process occurs repeatedly or continuously, with the process lasting for several weeks to months and even years.[1] The inflammation is a physiological process within the body; it can be identified by various symptoms, such as, severe pain, rheumatoid arthritis, and asthma. There are various standard anti-inflammatory drugs that are used to alleviate these symptoms, such as, non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. NSAIDs and corticosteroids inhibit the enzymes cyclooxygenase (COX) and phospholipase A2 (PLA2), respectively.[2] Non-selective NSAIDs inhibit not only COX2 but also COX1, activating the inhibition of prostacyclin and prostaglandin E2 (PGE2). The prostacyclin and PGE2 defend gastric mucosa in the stomach against exposure to stomach acid. The prolonged use of NSAIDs has various side effects, such as, nausea, vomiting, peptic ulcers, and gastric bleeding.[2] The selective COX inhibitors

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also cause various aftereffects, like increased risk of heart attack.[2,3] The corticosteroids have disadvantages, like the occurrence of resistance to corticosteroids.[3] NSAIDs have lots of side effects and corticosteroids when consumed in combination with herbal medicines.[4] There is a need for alternative anti-inflammatory drugs with minimum adverse effects, especially those derived from natural ingredients. However, the scientific report is not available on the estimation of its anti-inflammatory activity along with its propensity to inhibit lysis and increase the stability of the lysosome membrane, which is cognate to the red blood cells (RBC) membrane. The present research work was done to examine *in vitro* effect of methanolic and hydro methanolic extracts of *Z. officinale* and *Z. officinale* in combination with diclofenac sodium on hypotonicity-induced human RBC membrane.

Materials and Methods

Collection of Plant

The rhizomes of *Z. officinale* were collected from the Lakshmi Nagar market, Rajkot, Gujarat, India. Identification of the ginger was authenticated at the Department of Biosciences, Saurashtra University, and a voucher specimen (SU/DPS/Herbs/77) has been submitted in the departmental herbarium. The phytochemical screening of ginger rhizomes was performed. The chemical reagents used in the present research work were acquired from local suppliers.

Extraction Method and Preparation of Plant Extract

The rhizomes of ginger were dried in the shade and then pulverized to attain the powder. The 50 grams of dried powdered rhizomes of *Z. officinale* were extracted with methanol by the maceration process for 48 hours. The methanol solvent was volatilized and concentrated on a water bath at low temperature to get the crude extract, and for further use, the crude extract was stored in a desiccator. For the preparation of hydro-methanolic extract, the 50 grams powder was taken in 70 mL methanol and 30 mL distilled water and left for maceration for 48 hours, and after the maceration, the extract was filtered by using Whatman's filter paper. The acquired filtrate was stored for further research work.

HRBC Suspension Preparation

As per the article of Seema *et al.*, fresh blood was collected from humans who have not consumed NSAID's two weeks prior to the experiment, and the collected fresh blood was mixed with the sterilized Alsever solution. Alsever solution was prepared by adding 2% dextrose, 0.85% sodium citrate, 0.06% citric acid, and 0.44% sodium chloride in water. The centrifugation of blood was done at 3,000 rpm for 10 minutes, and sediment cells obtained after centrifugation were washed three times with iso-saline solution 0.85% having pH 7.2. The blood volume was measured and having been formed again as a 10% v/v suspension with iso-saline (Chaitanya *et al*., 2011).

Herbal Preparation

Ginger extract 25 µg was taken and dissolved in 10 mL of methanol, and after that, this is boiled for 10 minutes and cooled. After that, it was centrifuged for 10 minutes at 2,500 rpm, and after the centrifugation, the supernatant was collected and used for further study.

Preparation of Test Sample

Z. officinale (25 µg/10 mL), ginger with diclofenac sodium samples were prepared (12.5 + 12.5 µg = 25 µg/10 mL), respectively, wielding distilled water and to individual concentration 1 mL of phosphate buffer, 2 mL hyposaline, and 30 µL of HRBC suspension were added. The above mixture was incubated at 37°C for 30 minutes, and after that above mixture was centrifuged at 3,000 rpm for 25 minutes. In the supernatant solution, the hemoglobin content present was appraised spectrophotometrically at 570 nm. Diclofenac sodium (25 µg/10 mL) was used as a reference standard, and control was prepared by excluding the drug samples. The percentage inhibition of hemolysis or membrane stabilization was calculated according to the modified method described by Shinde *et al*.[13]

Membrane Stabilization Method

The HRBC membrane stabilization method was performed, as revealed by Sadique *et al*.[10] and Oyedepo *et al*.[7] In the suspension, the content of hemoglobin was adjudged by using ultraviolet-visible (UV) spectrophotometer at 570 nm.

Control Sample

0.03 mL stock erythrocyte + 5 mL hypotonic solution.

Test Sample

0.03 mL stock erythrocyte + 5 mL hypotonic solution containing herbal preparation (50–1,000 µg/mL).

Standard Sample

0.03 mL stock erythrocyte + 5 mL hypotonic solution containing diclofenac sodium (50–1,000 µg/mL).

The formula used for the calculation of % hemolysis of HRBC membrane is given below:

Percentage of hemolysis = (Test sample's optical density/Control sample's optical density) × 100

The formula used for the calculation of % protection of HRBC membrane is given below:

Percentage of protection = 100 - (Test sample's optical density/Control sample's optical density) × 100

Protein Denaturation Method

The protein denaturation method was performed, as revealed by Godhandaraman *et al*.[5] Mixture 0.2 mL egg albumin + 2.8 mL phosphate buffer saline pH 6.4 + 2 mL of ginger rhizomes extracts was incubated at 37 ± 2°C for

10 minutes and heated at 60°C for 10 minutes. At 640 nm in the UV spectrophotometer by using a vehicle as blank absorbance is assessed. Distilled water is used as a control. Diclofenac sodium with a 1 mg/mL concentration was used as a reference standard and prepared the same as a test solution for the measurement of absorbance.

The formula for the calculation of protein denaturation is given below:

Percentage of inhibition = (Absorbance of control - absorbance of sample)/Absorbance of control × 100

Results

The ginger powder was subjected to various standardization parameters, as shown in Tables 1 to 6, such as, ash value, extractive value, and phytochemical tests for hydro-methanolic and methanolic extract.

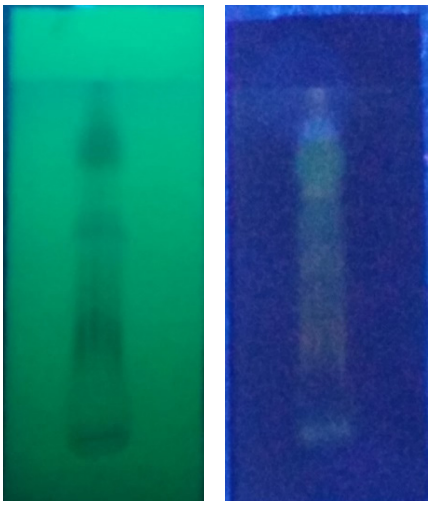
The mobile phase used was toluene:ethyl acetate (7:3), and four Rf values were found Rf1 = 0.48, Rf2 = 0.53, Rf3 = 0.71, and Rf4 = 0.79, respectively, as shown in Fig. 1. The inhibition of hypotonicity-induced HRBC lysis, i.e., stabilization of HRBC membrane, was taken as a measure of the anti-inflammatory activity. At different concentrations 50, 100, 200, 250, 500, and 1,000 mcg/mL for methanolic and hydro-methanolic extract of ginger (test), diclofenac sodium (standard), ginger, and diclofenac sodium (combination), the percentage of membrane stabilization was performed. It was noticed that 50 mcg/mL solution of methanolic extract of ginger, as well as, the combination of ginger and diclofenac sodium, was found to be most effective as compared to hydro-methanolic extract of ginger, as shown in Tables 7 and 8, and Figs 2 and 3.

Table 1: Ash values of methanolic extract of ginger

S. No.	*Ash values*	*Observation (%)*
1	Total ash	5.5
2	Acid insoluble ash	0.9
3	Water-soluble ash	1.2

Table 2: Ash values of hydro-methanolic extract of ginger

S. No.	*Ash values*	*Observation (%)*
1	Total ash	6.5
2	Acid insoluble ash	1.5
3	Water-soluble ash	4.6



UV 254 nm UV 366 nm

Fig. 1: TLC of *Z. officinale* at UV 254 and 366 nm

Table 3: Extractive values of methanolic extract of ginger

S. No.	*Extract types*	*Drug weight (gm)*	*Empty china dish weight (gm)*	*China dish weight with dry extract (gm)*	*Extractive values (%) (w/v)*
1	Alcohol-soluble extractive value	4	67.42	67.67	6.25
2	Water-soluble extractive value	4	68.24	68.8	16.5

Table 4: Extractive values of hydro-methanolic extract of ginger

S. No.	*Extract types*	*Drug weight (gm)*	*Empty china dish weight (gm)*	*China dish weight with dry extract (gm)*	*Extractive values (%) (w/v)*
1	Alcohol-soluble extractive value	4	64.21	64.5	7.25
2	Water-soluble extractive value	4	64.26	64.98	18

Table 5: Phytochemical tests for methanolic extract of ginger powder

S. No.	*Phytochemical tests*	*Observation*
1	Alkaloids	Positive
2	Glycosides	Positive
3	Tannins	Positive
4	Saponins	Positive
5	Carbohydrates	Positive
6	Gum and mucilage	Negative
7	Terpenoids	Positive
8	Flavanoids	Positive

Table 6: Phytochemical tests for hydro-methanolic extract of ginger powder

S. No.	*Phytochemical tests*	*Observation*
1	Alkaloids	Positive
2	Glycosides	Positive
3	Tannins	Negative
4	Saponins	Positive
5	Carbohydrates	Positive
6	Gum and mucilage	Negative
7	Terpenoids	Positive
8	Flavanoids	Positive

Table 7: *In vitro* anti-inflammatory activity of methanolic extract of *Z. officinale* on HRBC membrane hemolysis and membrane protection

Conc. (mcg/mL)	*% hemolysis of diclofenac sodium*	*% protection of diclofenac sodium*	*% hemolysis of ginger*	*% protection of ginger*	*% hemolysis of diclofenac sodium + ginger*	*% protection of diclofenac sodium + ginger*
50	68.66	31.34	13.66	86.34	13.57	86.43
100	40.8	59.2	22.86	77.14	29.46	70.54
200	23.48	76.52	51.25	48.75	37.23	62.77
250	12.77	87.23	69.29	30.71	48.48	51.52
500	8.84	91.16	82.59	17.41	62.23	37.77
1,000	7.41	92.59	88.13	11.88	68.48	31.52

Table 8: *In vitro* anti-inflammatory activity of hydro-methanolic extract of *Z. officinale* on HRBC membrane hemolysis and membrane protection

Conc. (mcg/mL)	*% hemolysis of diclofenac sodium*	*% protection of diclofenac sodium*	*% hemolysis of ginger*	*% protection of ginger*	*% hemolysis of diclofenac sodium + ginger*	*% protection of diclofenac sodium + ginger*
50	70.76	29.24	25.91	74.09	20.23	79.77
100	57.05	42.95	36.06	63.94	34.32	65.68
200	41.82	58.18	51.59	48.41	40.3	59.7
250	34.32	65.68	62.2	37.8	44.77	55.23
500	16.14	83.86	68.26	31.74	57.95	42.05
1,000	9.32	90.68	75.53	24.47	73.79	26.21

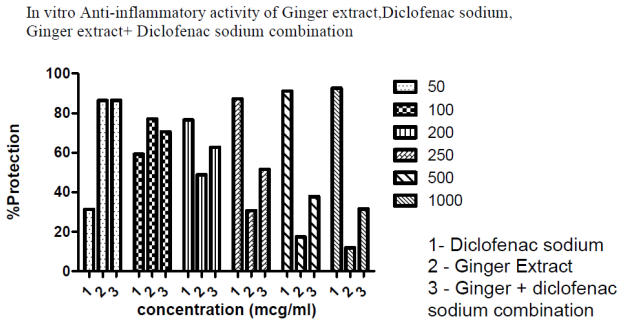


Fig. 2: *In vitro* anti-inflammatory activity of methanolic extract of ginger, diclofenac sodium, and combination of ginger with diclofenac sodium by HRBC method

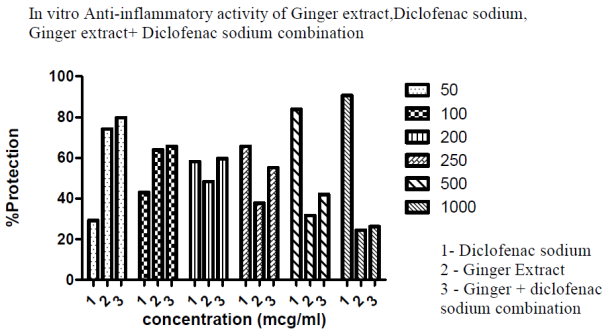


Fig. 3: *In vitro* anti-inflammatory activity of hydro-methanolic extract of ginger, diclofenac sodium, and combination of ginger with diclofenac sodium by HRBC method

In the case of standard, the % protection of membrane was found to increase with increasing concentration of diclofenac sodium. Inflammation is a reaction of living tissues towards injury. The steroidal anti-inflammatory agents will lysis and persuade the lymphocytes' re-apportion, which tenet expeditious and short term

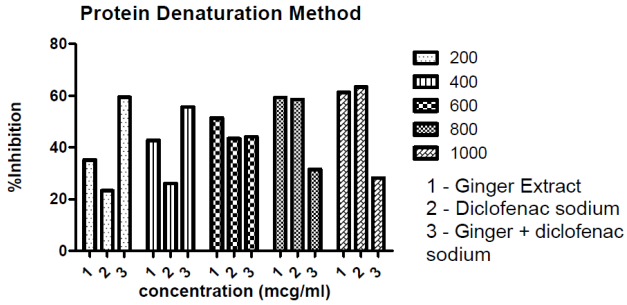


Fig. 4: *In vitro* protein denaturation method for hydro-methanolic extract of ginger, diclofenac sodium, and combination of ginger with diclofenac sodium

lessening of peripheral blood lymphocyte count to influence the longer phrase response.

The result stipulates that at various concentrations, the ginger extract will confer anti-inflammatory property. In the treatment of acute inflammation, ginger can be used as an efficacious therapeutic agent. The protein's denaturation is also responsible for anti-inflammatory activity. So, *in vitro* protein denaturation activity was performed for the methanolic, as well as, hydro-methanolic extract of ginger, diclofenac sodium, and for the combination of ginger with diclofenac sodium, which is shown in Tables 9 and 10, and Figs 4 and 5.

Discussion

The successive rhizome extract of *Z. officinale* manifested membrane stabilization effect by inhibiting hypotonicity induced lysis of the erythrocyte membrane. The erythrocyte membrane is similar to the lysosomal membrane, and the stabilization of the erythrocyte membrane manifests that the extract may also stabilize

Table 9: *In vitro* protein denaturation method for hydro-methanolic extract of ginger

	Concentration of plant extracts/ % protein denaturation				
Drug extracts	200	400	600	800	1,000
Ginger extract	35.18 ± 1.9	42.78 ± 3.39	51.42 ± 5.12	59.33 ± 1.98	61.35 ± 2.57
Diclofenac sodium (1 mg/mL)	23.27 ± 1.26	26.1 ± 1.46	43.45 ± 2.41	58.56 ± 2.54	63.37 ± 2.78
Ginger extract + diclofenac sodium (1 mg/mL)	59.45 ± 0.98	55.65 ± 2.34	44.15 ± 1.78	31.51 ± 1.33	28.23 ± 3.14

Table 10: *In vitro* protein denaturation method for methanolic extract of ginger

	Concentration of plant extracts/ % protein denaturation				
Drug extracts	*200*	*400*	*600*	*800*	*1,000*
Ginger extract	41.58 ± 1.73	56.87 ± 5.95	65.24 ± 4.35	71.45 ± 2.54	78.83 ± 5.17
Diclofenac sodium (1 mg/mL)	32.23 ± 1.21	41.42 ± 2.33	52.55 ± 1.99	65.67 ± 5.97	73.38 ± 2.31
Ginger extract + diclofenac sodium (1 mg/mL)	57.83 ± 2.23	52.37 ± 4.31	41.33 ± 1.11	36.66 ± 2.14	25.27 ± 1.76

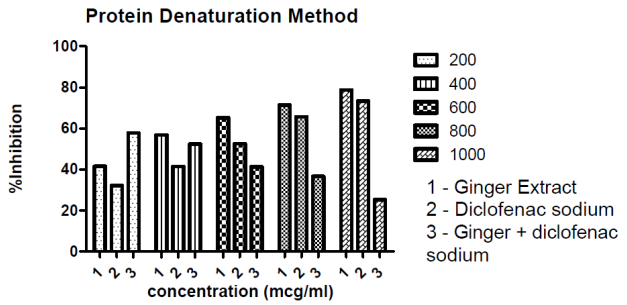


Fig. 5: *In vitro* protein denaturation method for methanolic extract of ginger, diclofenac sodium, and combination of ginger with diclofenac sodium

the lysosomal membrane.[14] Stabilization of the lysosomal membrane is important in releasing lysosomal constituents of activated neutrophils, such as, bacterial enzymes and protease, causing extra tissue inflammation and damage on extracellular release.[15] The particular mechanism by using the extract for the membrane stabilization is yet not known; hypotonicity-persuaded hemolysis may emerge by contraction of the cells because of the osmotic loss of fluid components, as well as, intracellular electrolyte. The extract may inhibit the processes, stimulating or enhancing the efflux of these intracellular components.[16,17] As a comparison to control on the basis of *in vitro* study, it was evaluated that hydro-methanolic extract of ginger showed significant anti-inflammatory activity. In flavonoids, anti-inflammatory effects have been noticed against different enzymes, such as, protein kinase C, protein tyrosine kinases, phospholipase A2, and phosphodiesterase flavonoids possess potent inhibitory activity. Protein denaturation is a well-reported cause of inflammation. There are various inflammatory drugs (salicylic acid, etc.), which have shown dose-dependent ability to thermally induced protein denaturation.[18] The denaturation is wielded loosely to identify the change of proteins from a soluble to an insoluble form turned out by a huge variety of chemical and physical agents, together with acids, alkalies, alcohol, acetone, and salts of heavy metals.[19] Various literature shows that protein denaturation is one of the origins of rheumatoid arthritis[20,21] because of auto-antigens' production in definite rheumatic diseases. The denaturation mechanism is entangled in changes of electrostatic force, hydrogen, hydrophobic, and disulfide bonds. The extracts may probably inhibit the liberation of the lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents incorporate bactericidal enzymes and proteinases, which on extracellular release cause extra tissue inflammation and damage.[3] Ginger showed significant anti-inflammatory activity as the concentration increases, so it is a good alternative for other synthetic anti-inflammatory agents when it is consumed alone, but when it is used in combination with diclofenac sodium, it showed good anti-inflammatory activity at lesser concentration and as the concentration increases the anti-inflammatory activity for the combination ginger with diclofenac sodium gets decreased.

Conclusion

It is concluded that if anyone accidentally or intentionally consume excess amount of herbal medicine with allopathic drug it may cause interaction and sometimes leads to adverse effects.

References

1. Chatterjee S, Banerjee A, Chandr. *Hemidesmus indicus*: A Rich Source of Herbal Medicine. Medicinal and aromatic plant. 2014;3(4):3–4. Available from: doi.org/ 10.4172/2167-0412.S3-e002.
2. Chaitanya S, Chippada SS, Volluri SR, Bammidi M. *In-vitro* anti-inflammatory activity of methanolic extract of *Centella asiatica* by HRBC membrane stabilisation. Biosciences Biotechnology Research Asia. 2011; 8(1). Available from: doi.org/10.13005/bbra/867
3. Chou CT. The anti-inflammatory effect of an extract of *Tripterygium wilfordii* hook F on adjuvant induced paw oedeme in rats. Phytotherapy research. 1997;11: 152-154.
4. Khemasili K, Widodo M Aris, Sanarto S, Setyawati K. In vitro and in vivo Anti-inflammatory Activities of *Coptosapelta flavescens* Korth Root's Methanol Extract, Journal. of Applied Pharm. sci. 2018; Available from: doi.org/10.7324JAPS.2018. 8907
5. Godhandaraman S, Ramalingam V. *In vitro* anti-inflammatory

activity of different parts of Pedalium murex (L.), International journal of herbal medicine. 2016; 4(3): 31-36.
6. Muhammad K N, Swandari P.Membrane Stabilization Activity as Anti-Inflammatory Mechanisms of *Vernonia amygdalina* leaves extracts, The international conference on tropical studies and its Applications 2017; November, Available from: doi.org/10.13140/RG.2.2.10064.76804
7. Oyedapo O. and Akinpelu A. Red blood cell membrane stabilizing potentials of extracts of *Lantana camra* and its fraction, International journal of plant physiology and biochem.2010;2(4): 46-51
8. Rajendran V. and Lakshmi K.S. *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplococso cochinchnensis* moore ssp *laurina*. Bangladesh journal of pharmacology. 2008; 3: 121
9. Rosa MD, Giround JP, Willoghby DA. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J pathol. 1971; Available from: doi.org/10.1007/BF00500909.
10. Sadique, J, W.A. Al-Rqobah, M.F. Bughaith and AR El-Gindy. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterrepia 1989;60: 525-532.
11. Sakat S, Juvekar AR, Gambhire MN. *In vitro* anti-oxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International journal of pharma and pharmacological Science. 2010;2(1):146-155.
12. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of anti-inflammatory activity of *Tephrosia purpurea* in rats. Asian Pacific journal of tropical medicine. 2010; 3(3):193–195.
13. 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. 1999; 70:251–7.
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*. International journal of Drug Development and Research. 2011; 3:124–127.
15. Yurugasan N, Vember S, Damodharan C. Studies on erythrocyte membrane IV: *In vitro* haemolytic activity of Oleander extract. Toxicology Letter. 1981; 8:33–8. Available from: doi.org/10.1016/0378-4274(81)90134-x.
16. Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp *Laurina*. Bangladesh Journal of Pharmacology. 2008; 3:121–4.
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. Chinese Journal of Natural Medicine. 2010; 8:285–92; Available from: doi.org/10.1186/s13104-015-1384-5
18. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology 1968; 20:169173; Available from: doi.org/10.1111/j.2042-7158. 1968.tb09718.x
19. Mann G. Chemistry of the proteids, London and New York.1906; 336-344.
20. Vane JR. Botting RM. New insights into the mode of action of anti-inflammatory drugs. Inflammation Research. 1995; 44(1): 1-10.
21. Mizushima Y. Screening test for anti-rheumatic drugs, Lancet. 1966; 2:443. Available from: doi.org/ 10.1016/S0140-6736(66)92756-5

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