International Journal of Engineering Sciences & Research Technology

(A Peer Reviewed Online Journal)
Impact Factor: 5.164

IJESRT

RESEARCHERID
THOMSON REUTERS

[Sireesha, *et al.*, 9(2): February, 2020]
IC™ Value: 3.00

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

PHYTOTHERAPEUTICAL EVALUATION OF CHRYSANTHEMUM FLOWERS FOR ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY

P.Sailaja Rao[1] & Sireesha K*[2]

[1]Dr. P Sailaja Rao, Assistant Professor, Department of Pharmacology, College of Pharmacy, Jazan University, Kingdom of Saudi Arabia.

[82]Dr. Sireesha K, Associate Professor, Department of Pharmacology, Sri Venkateshwara College of Pharmacy, Hyderabad, Telangana State, India.

DOI: 10.5281/zenodo.3653467

ABSTRACT

Inflammatory diseases are major cause of morbidity and mortality in the world. Inflammation is a complex mechanism employed by the body to promote healing and restoration to normal function in the event of injury. At the site of inflammation, overproduction of reactive oxygen species (ROS) predominates subsequent oxidative stress and potentially cause damage to biomolecules such as DNA, lipids and proteins. Now a days, search for natural entities with anti-inflammatory activity with lesser side effects is vital. Therefore, efforts are being continuously made to identify such agents and to validate their scientific authenticity. The main aim of this study is to evaluate the antioxidant and anti-inflammatory properties of chrysanthemum morifolium methanolic flower extract. Chrysanthemum morifolium is an important medicinal plant with many functions.Their effects were evaluated by carrageenan-induced paw edema and egg white induced paw edema method in experimental rats for 4 h. The oral administration of MECM (200 & 400 mg/kg) exerted potent antiinflammatory activity by carrageenan reducing paw edema (35 & 46.2%) and by egg white induced paw edema method (50.94 & 53%) respectively and it was comparable with the standard drug, Diclofenac sodium (10 mg/kg, 62% and 67%) at the end 4 hours.. In vitro antioxidant studies were studied by two methods i.e., hydrogen peroxide scavenging assay and reducing power assay. The IC_{50} values of the test extract were compared to the standard anti oxidant Ascorbic acid. Significant results were obtained ($p<0.01$). The obtained results therefore suggest the MECM is considered to be a valuable source of remedy for inflammation.

KEYWORDS: Carrageenan, Egg-white , Inflammation.

1. INTRODUCTION

Inflammation is a protective mechanism, which took place when the body responds to a kind of stimuli like infections, irritants or different cellular and tissue damages[1]. Inflammatory process are generally associated with changes in cellular status toward a more oxidized redox tone compared to a normal cell, a situation characterized by an increased production of reactive oxygen species (ROS) and accompanying oxidative stress[2]. Free radicals are essential to the successful functioning of our innate immune response but ROS, RNS, RSS conspire to carry out many other important cellular signaling function[3]. During the development of inflammation, the concentrated actions of molecular signaling determine whether inflammatory cells undergo migration, activation, proliferation, differentiation or clearence[4]. The mechanism of inflammation is attributed with the aid of the release of ROS from activated immune procedure, neutrophils and macrophages. Additionally, ROS proliferate inflammation through a stimulating release of cytokines comparable to interleukin-1, TNF-α, and interferon-γ, which stimulate extra neutrophils and macrophages. Free radicals are most important mediators, which provoke inflammatory process and their neutralization by antioxidants and radical scavengers can cut back inflammation[5]. At present available anti-inflammatory drugs, inhibit the product from cyclooxygenase (COX) enzymes, COX-1 and COX-2, which includes prostaglandins and thromboxane, common inflammatory mediators[6]. Both steroidal and Non-steroidal antinflammatory drugs (NSAID's), plays their role as a cure of irritation or inflammation through inhibiting these two enzyme. Long-term use of these medications results in gastric erosions and duodenal ulcers as well as renal toxicity.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

The World Health Organization (WHO) has estimated that more than 75% of the world's total population depends on herbal drugs for their primary healthcare needs[7] . Therefore, there is a major research emphasis on discovering plants with antioxidant and anti-inflammatory potential that may be treat various kinds of injuries or protect against diseases[8]. Chrysanthemum morifolium, a member of Asteracea family blooms during late summer and early autumn. *Chrysanthemum morifolium* Ramat. (Asteraceae) (CM) is a medicinal and edible plant that contains a wide variety of natural compounds, including polyphenols and volatile oils.

2. MATERIALS AND METHODS

2.1 Collection of Plant material and extraction[9]

The Flowers of *Chrysanthemum morifolium* were collected from a local market during the month of October 2018. This material was identified and authenticated by a botanist at SV University, Tirupati, Andhra Pradesh, India. The freshly collected flowers of the plant *Chrysanthemum morifolium* were cleared from dirt and then the flowers were dried under shade for about 10 days and coarsely powdered in a mixer grinder. The powdered material was stored for extraction process. The powder was subjected to methanol extraction, in Soxhlet apparatus and was run about 10 cycles. After filtration through Whatman filter paper, the filtrates were dried in desiccator.

2.2 Chemicals

Carrageenan was procured from Sigma Aldrich, Bombay, India. Diclofenac was a gift sample from Dr. Reddy's Laboratories, Omeprazole was procured from Aurobindo Pharma, Hyderabad. Aspirin was procured from Sigma labs, Mumbai. Acetic anhydrous, Ascorbic acid, chloroform, formalin and hydrogen peroxide were procured from SD Fine Chem Limited, Mumbai. Ammonia, carboxy methyl cellulose, ferric chloride and glacial acetic acid from Himedia laboratories, Mumbai.

2.3 Preliminary Phytochemical Screening[10]

The flowers of *Chrysanthemum morifolium* was screened for the presence of various phytoconstituents like alkaloids, flavanoids, steroids, tannins, glycosides, triterpenoids and saponins.

Tests carried out for different phytochemical constituents

1) *Test for alkaloids*: A small portion of crude extract was dissolved in 5 ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayer's reagent separately. Any precipitate or turbidity with the reagents suggested the presence of alkaloids.
2) *Test for flavonoids*: A few drops of conc. hydrochloric acid and 1-2 magnesium turnings were added to 1 ml of methanolic extract. The presence of flavonoids was indicated by the development of pink or magenta-red colour.
3) *Test for phenols (Ferric chloride test)*: A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.
4) Test for amino acids and proteins (1 % ninhydrin solution in acetone): 2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.
5) *Test for carbohydrates (Molisch test)*: To a fraction of extract α-naphthol and alcohol was added. It was mixed well and conc. Sulphuric acid was added drop by drop by keeping the test tube in inclined position. Violet ring is formed at the junction of two layers which shows the presence of carbohydrates.
6) *Test for saponins (Foam test):* To 2 ml of extract was added 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirmed the presence of saponins.
7) *Test for sterols (Liebermann-Burchard test)*: 2 ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red colour.
8) *Test for tannins (Braymer's test):* 2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Acute Toxicity Studies of METS[11]

Acute toxicity studies were carried out in order to check the toxic effects for methanolic extract of flowers of *Chrysanthemum morifolium*. The studies were performed as per Organization for Economic Cooperation and Development (OECD). The method is used to evaluate the acute oral toxicity is up and down procedure (OECD

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

guideline-425). Up and down procedure (OECD guideline-425) acute toxicity studies were carried out as per the OECD 425 guidelines.

3. ANIMALS

Animal Protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experimentation on Animals) through its reference no: IAEC/SVCP/2016/009, Dated: 27/2/16. Male Wistar rats, weighing (180-250 gms) were obtained from NIN (National Institute of Nutrition, Hyderabad. The animals were acclimatized to the experimental room at a temperature of 23±20 C, controlled humidity conditions (50-55%) and 12 hr light and 12 hr dark cycles. They were fed with standard food pellets (Hindustan Lever, Hyderabad) and water ad libitum.

4. EVALUATION OF ANTI-INFLAMMATORY ACTIVITY [12,13]

The *in vivo* evaluation of Anti-inflammatory activity of the methanolic extract of flowers of *Chrysanthemum morifolium* in

1. Carrageenan induced paw edema
2. Egg white induced hind paw edema

4.1 Carrageen an induced paw edema

Anti-inflammatory activity was carried out using carrageenan induced paw edema in rats, Group-I served as normal control, receives 0.9% normal saline solution (1ml/kg b. wt), group-II served as disease control received carrageenan (1% w/v) and saline, group-III received carrageenan (1% w/v) and MECM (200 mg/kg b. wt.), group-IV carrageenan (1% w/v) and MECM (400 mg/kg b. wt.) and group-V is served as a standard group received carrageenan (1% w/v) and Diclofenac sodium (10 mg/kg b. wt.) *p.o*. Each rat was administered *orally* with the respective drug 1 h prior to the administration of the carrageenan 0.05 mL of 1% carrageenan was injected aseptically into the sub-plantar surface of left hind paw of each rat. Paw edema was measured by mercury plethysmometer hourly up to 4 h to study the effect of aqueous extract the plant on inflammation. The difference between the '0' and '3rd' h reading gives the actual edema.[14]

Table 1: Study design for carrageenan induced paw edema model

S.No	Group	Treatment
1	Group – I	Control(0.9% normal saline)
2	Group – II	Disease control – carrageen an (1% w/v) and normal saline (1ml/kg b. wt.) *p.o.*
3	Group – III	T1 - MECM (200 mg/kg, b.wt.) *p.o.*+ Carrageenan (1% w/v)
4	Group – IV	T1 - MECM (400 mg/kg, b.wt.) *p.o.*+ Carrageenan (1% w/v)
5	Group – V	Standard– Receives Diclofenac sodium (10 mg/kg b. wt.) *p.o* + carrageenan (1% w/v)

4.2 Egg white induced hind paw edema:

Albino Wistar rats of either sex weighing about 150 - 180 g were divided into five groups of six animals each. Group I served as a Control group receives 1mL/kg vehicle (0.3% CMC), group-II served as disease control received egg albumin (0.1 mL) and 1mL/kg vehicle (0.3% CMC) *p.o.*, group-III received egg albumin (0.1 mL) and MECM (200 mg/kg b. wt) *p.o*., group-IV egg albumin (0.1 mL) and MECM (400 mg/kg b. wt) *p.o*. and group-V is served as a standard group received egg albumin (0.1 mL) and Diclofenac sodium (10 mg/kg b. wt.) *p.o*. All the drugs and vehicle were given 1 h prior to the study. Freshly taken egg white (0.1 ml) was injected into the sub plantar tissue of the left hind paw of the rat. The volumes of the injected paws were measured at 0, 1, 2, 3 and 4hrs using a plethysmometer. The percent increase in paw edema of the treated group was compared with that of the control and standard groups of the drugs were studied.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Table 2 Study design for Egg white induced hind paw edema

S. No	Groups	Treatment
1	Group – I	Control 1mL/kg vehicle (0.3% CMC)
2	Group – II	Disease control – egg albumin (0.1 mL) and1mL/kg vehicle (0.3% CMC) *p.o.*
3	Group – III	T1 - MECM (200 mg/kg, b.wt.) *p.o.* + Egg albumin (0.1 mL)
4	Group – IV	T1 - MECM (400 mg/kg, b.wt.) *p.o.* + Egg albumin (0.1 mL)
5	Group – V	Standard– Receives Diclofenac sodium (10 mg/kg b. wt.) *p.o* + egg albumin (0.1 mL)

5. ANTI OXIDANT ASSAY

Methanolic extract of flowers of *Chrysanthemum morifolium* (MECM) was tested for *in-vitro* antioxidant activity using standard methods. Amongst them, two methods were chosen for the present study. The absorbance was measured spectrophotometrically against corresponding blank solution. The percentage inhibition was calculated by the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{OD\,Control - OD\,Sample}{OD\,Control} x100$$

IC_{50} which is the concentration of sample required to scavenge 50% of the free radicals was calculated. IC_{50} was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

5.1 H_2o_2 Radical Scavenging Assay[15]

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

➢ ***Preparation of reagents***

1. **Phosphate buffer solution pH 7.4:** Add 250.0 ml of 0.2 M potassium dihydrogen phosphate to 393.4 ml of 0.1 M sodium hydroxide and make up the volume to 1000 ml with the distilled water.
2. **Potassium dihydrogen phosphate (0.2M) solution:** Potassium dihydrogen phosphate (2.72 g) was dissolved in distilled water and volume made upto 100 ml.
3. **Sodium hydroxide solution (0.1M) solution:** 0.4 g of sodium hydroxide was dissolved in distilled water and volume made up to 100 ml.

➢ ***Method***

A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (P^H 7.4) Test compounds (10–50 µg/mL) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

5.2 Reducing Power Assay[16]

This method was based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferriccyanide + Ferric Chloride ⟶ Potassium ferrocyanide + Ferrous Chloride

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

➢ ***Preparation of reagents***

- **Phosphate buffer pH 6.6:** Potassium dihydrogen phosphate (62.5 ml 0.2 M) was added to 250 ml volumetric flask and also 20.5 ml of 0.2 M NaOH and made up to volume 250 ml with distilled water.
- ***Phosphate (0.2 M) solution*:** Potassium dihydrogen phosphate (2.72 g) was dissolved in distilled water and volume made up to 100 ml.
- ***Sodium hydroxide solution (0.2 M) solution*:** 0.8 g of sodium hydroxide was dissolved in distilled water and volume made up to 100 ml .
- ***Potassium ferricyanide (1% w/v) solution*:** Potassium ferricyanide (1g) was dissolved in water and volume made up to 100 ml in volumetric flask.
- **Ferric chloride solution (0.1% w/v):** Ferric chloride (25 mg) was dissolved in distilled water and volume made up to 25 ml in volumetric flask.

➢ ***Method***

To 1 ml of test and standard compounds added 2.5 ml of potassium ferricyanide (1 % w/v), 2.5 ml of phosphate buffer (pH 6.6) and incubated at 50^0 C for 30 min. To 2.5 ml of above supernatant liquid, added 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ solution (0.1% w/v). The absorbance of ferric ferrous complex was measured using phosphate buffer pH 6.6 as control at 700 nm using UV-Visible spectrophotometer and estimated the increase in absorbance.

6. RESULTS

6.1 Preliminary Phytochemical Screening

The flowers of *Chrysanthemum morifolium* was screened for the presence of various phytoconstituents like alkaloids, flavanoids, steroids, tannins, glycosides, triterpenoids and saponins. The preliminary phytochemical investigation for methanolic extract of *Chrysanthemum morifolium* flower heads showed the presence of Alkaloids, steroids, flavonoids, triterpenoids, tannins & phenolic compounds, saponins, glycosides. The results were showed in Table 3.

Table No 3: Preliminary Phytochemical analysis for MECM flower heads

Phytoconstituents	**Results**
Alkaloids	+
Carbohydrates	_
Flavonoids	+
Triterpenoids	+
Saponins	+
Phenolic compounds	+
Glycosides	+

Note: + indicates present; - indicates absent

6.2 *In vivo* ANTIINFLAMMATORY ACTIVITY

Methanolic extract of *Chrysanthemum morifolium* flower heads was explored for its anti-inflammatory activity in Carrageenan induced paw edema and Egg white induced hind paw edema rodent models. All the results obtained in this study were included below.

6.2.1 Carrageenan induced paw edema

Table 4: Effect of MECM on Carrageenan induced paw edema

Treatment	**Change in paw volume (mL) at different hours**				
	0hr	**1hr**	**2hr**	**3hr**	**4hr**
Control	1.20±0.016	1.21±0.013	1.21±0.01	1.20±0.007	1.20±0.015

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

1% Carrageenan	1.22±0.02	1.61±0.009**	1.65±0.014**	1.69±0.018**	1.73±0.008**
MECM (200mg/kg)	1.65±0.022	1.60±0.01**A	1.48±0.004**bA (10%)	1.23±0.01**aA (27%)	1.12±0.021**aA (35%)
MECM (400mg/kg)	1.60±0.01	1.57±0.012**A	1.34±0.010**aA (18%)	1.22±0.021**aB (28%)	0.93±0.015**aB (46.24%)
Diclofenac sodium (10 mg/kg)	0.82±0.020	0.77±0.012**	0.70±0.022** (57.5%)	0.64±0.014** (60.01%)	0.69±0.006** (62.01%)

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were compared with control (** p<0.001, * p<0.05), Disease control(a p<0.001, b p<0.003) and standard (A p<0.001, B p<0.05).

The present study showed that Methanolic extract of *Chrysanthemum morifolium* was evaluated by using Carrageenan-induced paw edema. The anti-inflammatory activity evaluated by using a parameter - reduction in paw thickness. The methanolic extracts and standard drug given at dose of 200mg, 400mg and 10mg/kg. The methanolic extract 200mg/kg showed reduced paw thickness (mm) was 1.65±0.022, 1.60±0.01, 1.48±0.004, 1.23±0.01, 1.12±0.021 at 0, 1, 2, 3 and 4hrs. The methanolic extract 400 mg/kg showed reduction of paw thickness (mm) was 1.60±0.01, 1.57±0.012, 1.34±0.010,1.20±0.021,0.93±0.015 at 0, 1, 2, 3 and 4hrs. The standard drug showed reduced paw thickness (mm) was 0.82±0.020, 0.77±0.012, 0.70±0.022, 0.64±0.014, 0.69±0.00 at 0, 1, 2, 3 and 4hrs. The maximum activity of the standard drug Diclofenac sodium was observed at 4th hr. but the reduction of thickness of the paw was evident from 2hrs onwards. The extracts 400mg/kg and 200mg/kg have showed reduced paw thickness was evident from 2hrs onwards, but the amount of diminution of the inflammation was less when compared to the standard group.

6.2.2 Egg white induced hind paw edema

Table 5: Effect of MECM on Egg white induced hind paw edema

Treatment	**Change in paw volume (mL) at different hours**				
	0hr	**1hr**	**2hr**	**3hr**	**4hr**
Control	1.23±0.011	1.25±0.009	1.24±0.005	1.24±0.010	1.24±0.007
Egg White	1.26±0.008	1.52±0.021**	1.71±0.009**	1.81±0.024**	2.12±0.020**
MECM (200mg/kg)	1.42±0.008	1.39±0.016**b	1.28±0.009** aA (25.14%)	1.19±0.005**aA (34%)	1.04±0.007**aB (50.94%)
MECM (400mg/kg)	1.41±0.513	1.33±0.012**bB	1.26±0.017**aA (26%)	1.11±0.010**B (38%)	0.98±0.015*a (53%)
Diclofenac sodium (10 mg/kg)	0.82±0.010	0.77±0.014**	0.70±0.013** (59%)	0.64±0.022** (64%)	0.69±0.013** (67%)

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by usingANOVA followed by Dunnett's test. Results were compared with control (** p<0.001, * p<0.01), Disease control(a p<0.001, b p<0.01)and standard (A p<0.001, B p<0.05).

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

The present study showed that Methanolic extract of *Chrysanthemum morifolium* was evaluated by using egg white-induced paw edema. The anti-inflammatory activity evaluated by using a parameter - reduction in paw thickness. The methanolic extracts and standard drug given at dose of 200mg, 400mg and 10mg/kg. The methanolic extract 200mg/kg showed reduced paw thickness (mm) was 1.42±0.008, 1.39±0.016, 1.28±0.009, 1.19±0.005, 1.04±0.007 at 0, 1, 2, 3 and 4hrs. The methanolic extract 400 mg/kg showed reduction of paw thickness(mm) was 1.41±0.51, 1.33±0.012, 1.26±0.017,1.11±0.010,0.98±0.015 at 0, 1, 2, 3 and 4hrs. The standard drug showed reduced paw thickness (mm) was 0.82±0.010, 0.77±0.014, 0.70±0.013, 0.64±0.022, 0.69±0.013 at 0, 1, 2, 3 and 4hrs. The maximum activity of the standard drug Indomethacin was observed at 4th hr. but the reduction of thickness of the paw was evident from 2hrs onwards. The extracts 400mg/kg and 200mg/kg have showed reduced paw thickness was evident from 2hrs onwards, but the amount of diminution of the inflammation was less when compared to the standard group.

Carrageenan has been widely used as a most harmful agent which has a property to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. Similarly, egg white also plays a role in inducing inflammation in experimental animals. These physiological agents when induced locally into the rats paw, produced a severe inflammatory reaction.

The phytochemical analysis of the plant extract using methods described indicated the presence of steroids, triterpenoids, alkaloids, flavonoids, tannins and saponins. The earlier studies had indicated the use of egg-albumin as a physiological agent in causing edema in rat hind paw. Carrageenan- induced rat paw edema and egg white induced hind paw edema methods are suitable for screening agents for anti-inflammatory activity which are frequently used to assess the anti-edematous effect of natural products.[17,18] Many investigations have proven that varieties of flavonoid molecules possess anti-inflammatory activity in various animal models of inflammation. Especially, some flavonoids were found to inhibit chronic inflammation of several experimental animals models. Quercetin results in decreased oxidative injury. Quercetin in particular is known for its iron-chelating and iron-stabilizing properties. Direct inhibition of lipid peroxidation is another protective measure.[19]

Acute inflammation is produced when water and plasma increases in tissues during arachidonic acid metabolism via cyclooxygenase and lipo-oxygenase enzyme pathways. It has two phases: 1st phase (begins immediately after Carrageenan injection and lasts for 1 hr) is characterized by release of histamine and serotonin; and 2nd phase (begins after 1 hr and lasts for 4 hr) is characterized by bradykinin release by prostaglandin mediator pathways. During repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are basic sources for forming a highly vascularised reddish mass termed as granulation tissue.[20] Egg albumin has been known to cause inflammation by inducing the release of two inflammatory mediators which are basically histamine and serotonin, which the fractions inhibited to reduce inflammation.[21]Few years back, highly effective class of novel anti-inflammatory drugs such as Celecoxib, Rofecoxib, and Valdecoxib etc. were introduced in the pharmaceutical market but unfortunately most of them were withdrawn from the market on account of their serious cardio functioning side effects, especially in high sensitive patients like pregnant women, new born children, elderly people etc. Worldwide, there is an increasing concern in finding new anti-inflammatory remedies not only having improved therapeutic index but also harmless. The results of the COX inhibition studies focus the importance of selected botanicals as an important resource for the isolation and identification of new COX-2 selective anti-inflammatory agents.[22]

The outcome of our study of and from the previous database on this plant, it can be predictable that the anti-inflammatory effect exerted is because of flavonoid content. The anti-inflammatory activity of MECM via edema inhibition indicates a lot-massive effect in comparison with Diclofenac sodium. Carrageenan-induced paw edema model is the most suitable experimental animal model for evaluating or screening the anti-inflammatory effects from natural products.[23]

6.3 Invitro antioxidant studies

6.3.1 Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging activity of MECM flower was evaluated and compared with Ascorbic acid and the results are given in table 6. The percentage inhibition (% inhibition) at various concentration (10-50

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

µg/ml) of MECM as well as standard Ascorbic acid (10-50 µg/ml) were calculated. The IC_{50} values are calculated and were found to be 69 µg/ml (Ascorbic acid) and 64.1 µg/ml MECM.

6.3.*2 Reducing power assay*

The reducing abilities of MECM flower was evaluated and compared with ascorbic acid and the results are given in table 7.The mean of three absorbance at various concentrations (10-50 µg/ml) of MECM as well as standard Ascorbic acid (10-50 µg/ml) were calculated. The reductive capabilities were found to increase with increasing of concentration of MECM as well as standard ascorbic acid. Maximum absorbance of the test extract was found to be 1.52±0.07 at 50 µg/ml concentration and 1.83±0.03 at 50 µg/ml of Ascorbic acid. The results were found to be significant when compared with ascorbic acid (std).

Table 6: Effect of MECM on Hydrogen peroxide scavenging assay

S. No	Test extract	Dose (µg/ml)	% inhibition (Mean±SEM)	IC_{50}value (µg/mL)
1	Ascorbic acid (Standard)	10	38.24 ± 0.26	
		20	48.4 ± 0.37	
		30	72.8 ± 0.74	69±0.01
		40	88.7 ± 0.70	
		50	92.6 ± 0.59	
2	Test Extract	10	32.9 ± 0.98	
		20	47.6 ± 0.72	
		30	63.3 ± 0.34	64.1±0.07*
		40	78.7± 0.95	
		50	86.6 ± 0.56	

*p<0.01 considered as significant; compared with corresponding standard

Table 7: Absorbance of test extract and standard Ascorbic acid at various concentrations in Reducing Power Assay

S. No	Test extract	Dose (µg/ml)	Absorbance (Mean±SEM)
1	Ascorbic acid (Standard)	10	0.39 ± 0.01
		20	0.48 ± 0.03
		30	1.09 ± 0.04
		40	1.48 ± 0.02
		50	1.83 ± 0.03
2	Test extract	10	0.23 ± 0.01
		20	0.38± 0.02*
		30	0.62± 0.04*
		40	1.13± 0.08*
		50	1.52± 0.07*

*p<0.01 considered as significant; compared with corresponding standard

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

7. DISCUSSION

Free radicals are identified to liable for a broad variety of pathological conditions. Antioxidants help body immune system to oppose those free radicals and protect itself, they do that either by scavenging free radicals or defending the defense mechanism[24]. Phenolic compounds are categorized in a few class; amongst them flavonoids are regarded as a most robust antioxidants. Flavonoids naturally occur in herbs and they have constructive results on human well-being. Past acknowledgement on flavonoid derivatives, states that it plays a great function in the efficiency of distinct plants antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities.[25,26] In a similar way, the molecular antioxidant response of phenolic compounds varies remarkably, depending on their chemical constitution.[27] In our present study with methanolic extract of *Chrysanthemum morifolium*, it has confirmed the prescence of phenolic compounds. It has shown tremendously bigger percentage of inhibition. As a result, from this study we can make a proposal, that the plant extract contain distinctive phytochemical components which are able of donating hydrogen to a free radical to lessen, either complete or partial, the vulnerability of injury. It has been said that presence of certain flavonoids exerts profound anti-inflammatory activity by stabilizing the lysosomal membrane.[28,29] The anti-inflammatory activity of *Chrysanthemum morifolium* extracts via edema inhibition indicates a lot-massive effect in comparison with Diclofenac sodium. Carrageenan-induced paw edema model is a suitable experimental animal model for evaluating or screening the anti-inflammatory effects from natural products.

8. CONCLUSION

In the present investigation, we have revealed that the methanolic extract of *Chrysanthemum morifolium* has strong antioxidant and anti-inflammatory properties, which supports the usual use of this plant as well as its prior findings. The anti-inflammatory activity exerted with the aid of this extract also thought to be correlated with its anti-oxidant property. Hence reasearch of this plant extract to establish the presecnce of chemical constituents is considered to take this reasearch work to the next step.

REFERENCES

[1] Oyedapo OA, Adewunmi CO, Iwalewa EO, Makanju VO. Analgesic, antioxidant and anti-inflammatory related activities of 2'-hydroxy-2, 4'-dimethoxychalcone and 4-hydroxychalcone in mice. J Biol Sci. 2008;8(1):131-6.
[2] Okin, D. and Medzhitov, R. Evolution of inflammatory diseases. Curr. Biol. CB.,22 (17), 733-740.
[3] Andrew Cumpstey and Martin Feelisch . Free radicals in inflammation. Molecular and cellular Mechanisms to the Clinic, first Edition. 2018: 695-717.
[4] Mohini P, Parag K, Shailesh K, Meghana L, Sanlhah B, Pravin C. Evaluation of anti-inflammtory activity of herbal gel formulation. J.Nat.prod. Plant Resource; 2011;1(2):25-28.
[5] Marín-Loaiza JC, Nieto-Camacho A, Céspedes CL. Antioxidant and anti-inflammatory activities of Pittocaulon species from Mexico. Pharmaceutical biology. 2013 Feb 1;51(2):260-6.
[6] Dinarello CA. Anti-inflammatory agents: present and future. Cell. 2010 Mar 19;140(6):935-50.
[7] Organization WHO (WHO traditional medicine strategy 2002–2005 Geneva: World Health Organization; 2002.
[8] Huang SS, Chiu CS, Lin TH, Lee MM, Lee CY, et al. Antioxidant and anti-inflammatory activities of aqueous extract of Centipeda minima. J Ethnopharmacol. (2013); 147: 395–405.
[9] Beninger C W, Abou-Zaid M M, Kistner A L E. A flavanone and two phenolic acids from Chrysanthemum morifolium with phytotoxic and insect growth regulating activity. Journal of Chemical Ecology, 2004; 30 (3): 589– 606.
[10] Kokate C K, Purohit A P, Gokhale S B. Text Book of Pharmacognosy. 33th Ed. *Nirali Prakashan*, 2001; Pune; 593-597.
[11] OECD, 425: Acute oral toxicity-fixed dose procedure. OECD Guidelines for Testing of Chemicals Section. 2001:1–14.
[12] Zamora Rodríguez Z B, González Álvarez R, Guanche D. Antioxidant mechanism is involved in the gastroprotective effects of ozonized sunflower oil in ethanol-induced ulcers in rats. Mediators of Inflammation. 2007. 65873.
[13] Cabral B, Siqueira E M S, Bitencourt M A O. Phytochemical study and anti-inflammatory and antioxidant potential of Spondias mombin leaves. Revista Brasileira de Farmacognosia. 2016; 26(3): 304–311.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

[14] Kalabharathi HL, Suresha RN, Pragathi B, Pushpa VH, Satish AM. Anti inflammatory activity of fresh tulsi leaves (Ocimum Sanctum) in albino rats. International Journal of Pharma and Bio Sciences. 2011;2(4):45-50.
[15] Arunachalam, G., Subramanian N., Pazhani G, P., Ravichandran V. (2009), 'Anti-inflammatory activity of methanolic extract of *Eclipta prostrate L.* (Astearaceae)'. *African journal of pharmacy and pharmacology*, 3: 97-100.
[16] Kalabharathi HL, Suresh RN, Pragathi B, Pushpa VH, Satish AM.'Anti-inflammatory activity of fresh tulsi leaves (Ocimum sanctum) in albino rats'. International Journal of Pharma and Bio Sciences.2011; 2(4):45–9.
[17] Akah PA, Nwambie AI. Evaluation of Nigerian traditional medicines: 1. Plants used for rheumatic (inflammatory) disorders. Journal of Ethnopharmacology. 1994 May 1;42(3):179-82.
[18] Amos S, Chindo B, Edmond I, Akah P, Wambebe C, Gamaniel K. Anti-inflammatory and anti-nociceptive effects of Ficus platyphylla extract in mice and rats. Journal of herbs, spices & medicinal plants. 2002 Feb 4;9(1):47-53.
[19] Elango V, Oliver C, Raghu PS. Antiulcer activity of the leaf ethanolic extract of Mimosa pudica in rats. Hygeia-Journal for Drugs and Medicine. 2012;4(1):34-40.
[20] Aziz MA, Sarkar KK, Roy DN. Acute toxicity study and evaluation of anti-inflammatory & CNS depressant activities of Richardia scabra. Pharmacologyonline. 2015 Dec 30;3:70-5.
[21] Nwidu LL, Airhihen B, Ahmadu A. Anti-Inflammatory and Anti-Nociceptive Activities of Stem-Bark Extracts and Fractions of Carpolobia Lutea (Polygalaceae). Journal of basic and clinical pharmacy. 2016 Dec;8(1):25.
[22] Shaikh RU, Pund MM, Gacche RN. Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system in vitro as well as in vivo. Journal of traditional and complementary medicine. 2016 Oct 1;6(4):355-61.
[23] Azam S, Huda AF, Shams K, Hasan PA, Mustafa KM, Hasan MM. Anti-inflammatory and anti-oxidant study of ethanolic extract of Mimosa pudica. Journal of Young Pharmacists. 2015 Jul 1;7(3):234.
[24] M. Umamaheswari, T.K. Chatterjee. In vitro antioxidant activities of the fractions of Coccinia grandis l. leaf extract. African Journal of Traditional, Complementary and Alternative Medicines. 2008; 5
[25] G. Di Carlo, N. Mascolo, A.A. Izzo, F. Capasso. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sciences. 1999; 65 : 337-353
[26] P. Montoro, A. Braca, C. Pizza, N. Detommasi. Structure?antioxidant activity relationships of flavonoids isolated from different plant species. Food Chemistry. 2005; 92 : 349-355 .
[27] N. Saeed, M.R. Khan, M. Shabbir. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine. 2012; 12 : 221 .
[28] O. Oyedapo, B. Akinpelu, K. Akinwunmi, M. Adeyinka, F. Sipeolu. Red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. Int J Plant Physiol Biochem. 2010; 2 : 46-51.
[29] M.A. Read. Flavonoids: naturally occurring anti-inflammatory agents. The American journal of pathology. 1995; 147 : 235.