ejpmr, 2017,4(12), 96-102

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

ANTI-INFLAMMATORY AND CYCLOOXYGENASES INHIBITORY EFFECTS OF *ASTERACEAE*, RICH IN FLAVONOIDS AND TANNINS

Ibrahim M. El-Ashmawy*

Professor, Department of Veterinary Medicine, College of Agricultural and Veterinary Medicine, Al Qassim University, Buraydah, Kingdom of Saudi Arabia and Department of Pharmacology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.

***Corresponding Author: Ibrahim M. El-Ashmawy**

Professor, Department of Veterinary Medicine, College of Agricultural and Veterinary Medicine, Al Qassim University, Buraydah, Kingdom of Saudi Arabia and Department of Pharmacology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.

Article Received on 11/10/2017 **Article Revised on 02/11/2017** **Article Accepted on 22/11/2017**

ABSTRACT

The species of *Asteraceae were* used traditionally in treatment of inflammatory disorders. The present study aimed to assess the anti-inflammatory effect of methanolic extract of aerial parts of *Rhanterium epapposum* Oliv. (*R.epapposum*), *Achillea fragrantissima* (Forssk.) Sch.Bip (*A. fragrantissima*), *Artemisia herba-alba* Asso. (*A.herba-alba*), *Echinops spinosus* L. (*E. spinosus*) and *Echinops hussonii* Boiss. (*E.hussonii*). The extracts were evaluated using the carrageenan-induced rat paw edema (Acute and sub-acute models), and turpentine oil –induced granuloma pouch bioassay. The efficacy against cyclooxygenases (COX-1 and COX-2) and their phytoconstituents were also investigated. The plant extracts of *A. fragrantissima*, *E. spinosus* and *A. herba- alba* exhibited marked anti-inflammatory activity in the both phases of carrageenan induced acute edema test. Moreover, the activity in carrageenan induced sub-acute edema test for the extracts of *A. herba-alba*, *E. spinosus* and *A. fragrantissima* in a dose-dependent manner. Meanwhile, the moderate anti-inflammatory activity obtained by the extracts of *E.hussonii* and *R.epapposum* in the edema induced by the sub-plantar injection of carrageenan (either at the acute or sub-acute inflammatory models). The plant extracts of *A. herba-alba*, *E. spinosus* and *A. fragrantissima* exhibited potential inhibitory action on exudate formation and the moderate activity induced by the extracts of *E.hussonii* and *R.epapposum*. It was observed that the extracts showed pronounced inhibitory effects against COX-1 and COX-2. This study proved that these plant extracts could be used for the treatment of rheumatism and other inflammatory disorders.

KEYWORDS: Anti-inflammatory, *Asteraceae*, Carrageenan, Turpentine oil, COX-1, COX-2.

1. INTRODUCTION

Anti-inflammatory drugs are currently used for medications, including pain, edema, inflammation, osteoarthritis, rheumatoid arthritis and skeletal muscle disorders.[1] Prolonged use of these drugs commonly induced severe, side effects.[2] Therefore, alternative agents with less severe side-effects are needed and botanical products can be important candidates.[3] Accordingly the need arise to develop new anti-inflammatory agents with minimum side effects.[4]

Asteraceaeous plants were distributed throughout the world, especially at the Kingdom of Saudi Arabia. The species of *Asteraceae*,a source of many biologically active compounds due to their bio-active properties, plants from *Asteraceae* family were commonly used in treatment of various diseases.[5-10] *R. epapposum* was used in local traditional medicine for skin infections and pain of gastrointestinal disturbances[11] and as an insecticide.[12] Additionally, authors[13,14] reported the antioxidant activity and the presence of flavonoids, tannins, sterols, triterpenes and essentioal oils in *R. epapposum*.This study described for the first time the anti-inflammatory effects of *R. epapposum*. *A. fragrantissima* is rich in polyphenolics, tannins, and flavonoids were widely used in traditional medicine for inflammations, gastrointestinal disorders, antiparasitic, antiviral, insecticidal and antiseptic to various infections for the urinary tract.[10,15] *A. herba-alba* was used traditionally, for the treatment of inflammations, bacteria, fungi, diabetes, intestinal worms, wounds, and diarrhea which were enriched in polyphenolics, tannins and flavonoids.[6-10] *E. spinosus, E.hussonii* and similar species traditionally, were used for antimicrobial, antiprotozoal, hepatoprotective, antiulcerogenic activity, anti-inflammatory, diuretic action, analgesic activity, heart pain, kidney disease, and diarrhea.[6-8,10]

Based on traditional use of some plants of this family in treating inflammatory disorders so, we decided to investigate the effects of methanolic extract of aerial parts of *R. epapposum*, *A. fragrantissima*, *A. herba- alba*,

E. spinosus and *E. hussonii* on different inflammatory models, in vitro cyclooxygenases inhibitory activity and the study was extended to determine their phytoconstituents.

2. MATERIALS AND METHODS

***2.1. Animals**:* Wister albino mature male rats (180±20 g) were obtained from the Animal House of the College of Agriculture and Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia and housed at a temperature of 22 - 28 ºC and relative humidity of 50–60%, with artificial light from 5.00 a.m. to 4.00 p.m. Animals had free access to tap water and standard rat chow, used for the study. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication no. 85-23, revised 1996). The local ethics committee approved the study.

2.2. Chemicals: Analytical grade chemicals were purchased from Sigma Aldrich, St. Louis, MO, USA and were used for the bioassays in the laboratory.

***2.3. Preparation of plant materials**:* The plants were collected at the flowering stage, in and around Al-Gouf and Al-Qassim Districts, Kingdom of Saudi Arabia. The collected plant species were identified and confirmed by Dr. A. Al- Sakeer, Department of Botany, College of Agricultural and Veterinary Medicine, Qassim University, Al Qassim, Kingdom of Saudi Arabia and a voucher specimen was deposited in the Department of Botany, for further reference. Shade dried and powdered plant materials were successively extracted as the following. About 300 gm of the powdered plant were soaked in 3000 ml methanol. It was left for 72 h. with intermittent shaking till obtain methanol extract. The methanol extract was filtered using Whatman No.1 filter paper and the residue was concentrated until obtaining paste under vacuum using the rotary evaporator (Rota vapor R-215, Bǖtchi, Switzerland). The extracts were used for evaluation of the anti-inflammatory activity and phytochemical constituents. Respectively, the voucher specimens (1005, 1006, 1007, 1008 & 1009) for the methanolic extract of aerial parts of *R. epapposum*, *A. fragrantissima*, *A. herba- alba*, *E. spinosus* and *E. hussonii* were deposited at herbarium unit at the Department of Veterinary Medicine, College of Agricultural and Veterinary Medicine, Al Qassim University, Buraydah, 51452 P.O. 6622. Kingdom of Saudi Arabia.

***2.4. Phytochemical analysis: Total phenolics and total tannins content**:* The methods of Singleton and Rossi[16] was applied using Folin-Ciocalteu's reagent, with some modifications, in which few amount of each residue (50 mg) was mixed with 2.5 ml of deionized water followed by the addition of 0.25 ml of Folin-Ciocalteu's reagent and allowed to react for 6 min. To measure the absorbance at 765 nm (using spectrophotometer, SPECTRO 22, USA) 2.5 ml of sodium carbonate 7% was added and allowed to stand for 1 hr. Measurements were calibrated to a standard curve of prepared Gallic acid solution, and the total phenolic was expressed as mg Gallic acid equivalent per g of residue. Total tannin in the extracts was determined by a modification of the Folin-Ciocalteu method using polyvinyl poly pyrrolidone (PVPP) to separate tannin-phenols from non-tannin phenols.[17] About 100 mg of PVPP was added to 1ml of sample extract diluted in 1ml of water and left 15 min at 4°C. After centrifugation 5000 rpm for 10 min, PVPP forms a precipitate with tannins, and the supernatant has only simple phenols. Simple phenols were determined using the Folin-Ciocalteu reagent as previously described above. The difference between total and simple phenol values represents the total tannin content, expressed as mg Gallic acid equivalents g residue.

2.5. Total flavonoids: The method of Zhishen et al.[18] was used to measure the total flavonoids content. A known weight of extract residue dissolved in 1 ml methanol was added to 5 ml distilled water. At zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the 15 ml capacity volumetric flask, then after 5 min, 0.6 ml of 10% (w/v) aluminum chloride ($AlCl_3$) was added and, after 6 min, 2 ml of 1M Na OH were added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm (using spectrophotometer, SPECTRO 22, USA) against the blank (water) and flavonoid content was expressed as mg quercetin equivalents/ g residue.

2.6. Evaluation of the anti-inflammatory activity: The carrageenan-induced rat paw edema model (Acute inflammatory Model): The methods of Winter et al.[19] and Adeyemi et al.[20] were adopted. A total of twelve groups of rats having six animals each were used for the study. In the control group, only the vehicle, 0.5% carboxy methyl cellulose Na (CMC), was administered. Positive control groups were treated with diclofenac Na (20 mg/kg body weight) as the reference drug. Each extract was given in two doses level (200 and 400 mg/kg b. wt.). The test extracts/standard drug, diclofenac Na were suspended in 0.5% CMC and administered orally in a volume of 1 ml/100 g body weight of animal, once daily with the help of an oral gastric tube. Details of dose, time of administration, and duration of treatment were mentioned for each experimental paradigm studied. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of freshly prepared solution of 1% of carrageenan (Sigma Aldrich, St. Louis, MO, USA) in sterile 0.9% normal saline was injected into the sub plantar region of the left hind paw under light ether anesthesia. An equal volume of saline was injected into the right hind paw and served as internal control for the degree of inflammation in the left hind paw. The paw edema was measured Plethysmographically (Ugo Basile 7150, Varese, Italy Plethysmograph) and re-measured again 1, 2 and 4 h after injection of carrageenan. Edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition (or percent protection

against inflammation) for each rat and each group was calculated according to the following equation.

$$\% \text{ Inhibition} = \frac{(Vt-Vo)\text{ control} - (Vt-Vo)\text{ test compound}}{(Vt-Vo)\text{ control}} \times 100$$

Where Vt is the mean volume of edema at specific time interval and Vo is the mean volume of edema at zero time intervals.

2.7. Sub-acute inflammatory model: Rats in the first experiment were given the same test compounds in two doses level (200 and 400 mg/kg b. wt.) daily for 7 successive days. A solution of carrageenan (1%, 0.1 ml) was injected into the sub planter region of the left hind paw under light ether anesthesia 1 h after oral administration of the test material. A second injection of carrageenan (1%, 0.1 ml) was given on the third day. The changes in the volume of paw were measured Plethymographically at the first and eighth days.[19,20]

2.8. Turpentine oil–induced granuloma pouch bioassay: This chronic inflammatory model was performed as previously described[21] and modified using turpentine oil as irritant.[22] In ether-anaesthetized rats subcutaneously dorsal granuloma pouch was made by injecting 2 mL of air, followed by injecting 0.5 mL of turpentine oil into it. As mentioned previously plant extracts given orally (at a doses level 200 and 400 mg/kg b. wt.) one h before turpentine oil injection and continued for seven consecutive days. On the eighth day, the pouch was opened under light ether anesthesia and the exudates were collected by a syringe. The volume (mL) of the exudates was measured and the percentage inhibition of inflammation relative to the control was determined as follows: % inhibition = (V control- V treated) / V control x 100.

2.9. Determination of in vitro COX-1 and COX-2 inhibitory assay: This was approved for the plant extracts showed potent anti-inflammatory effects in animal models using Cayman colorimetric COX (ovine) inhibitor screening assay kit provided by Cayman chemicals, USA. Inhibitor screening assay employs the peroxidase component of cyclooxygenase. The peroxidase activity was assessed colorimetrically by monitoring the appearance of oxidized N, N, N, N-tetra methyl-p-phenylenediamine (TMPD) at 590 nm. Diclofenac Na, the non selective COX-2 inhibitor and celecoxib, the selective COX-2 inhibitor were used as a positive control. The analyses were done in duplicate and calculations of the percent of inhibition were directed as described by the Cayman Chemical Company Protocol.[23]

***2.10. Statistical analysis**:* Data were submitted to One Way ANOVA, $p \leq 0.05$. When a significant difference was found, the means were compared using Duncan, multiple range test.[24] Calculations were carried out using the SAS system.[25]

3. RESULTS AND DISCUSSION

In the present study, the plant extracts of *A. fragrantissima*, *E. spinosus* and *A. herba -alba* exhibited marked anti-inflammatory activity in the both phases of carrageenan induced acute edema test in a dose-dependent manner (Table 2). But the strongest activity of these extracts were arranged as follows, *A. herba-alba*, *E. spinosus* and *A. fragrantissima* against carrageenan induced sub-acute edema test also in a dose-dependent manner (Table 3). The moderate anti-inflammatory activity induced by the extracts of *E.hussonii* and *R. epapposum* in the edema induced by the sub-plantar injection of carrageenan (either at the acute or sub-acute inflammatory models). Inflammation is the integral part of body‘s defense mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes. While the features of chronic inflammation includes infiltration of mononuclear cells, proliferation of fibroblasts, blood vessels and increased connective tissue formation.[26] Anti-inflammatory drugs inhibit different stages of inflammation.[27]

The anti-inflammatory effect of methanol extract of aerial parts of *R. epapposum* and *A. fragrantissima, A. herba–alba, E. spinosus* and *E. hussonii* was assessed in different inflammatory models. Carrageenan- induced inflammatory edema in the hind paw of rats. Inhibition of carrageenan-induced inflammation in rats is one the most suitable test procedures to monitor anti-inflammatory agents.[28] Carrageenan, a mucopolysacharide derived from Irish Sea moss Chondrus, was used to induce experimental arthritis. Carrageenan was non-antigenic and did not produce any systemic effects.[29] Carrageenan produces acute inflammation believed to be biphasic; the early phase (1-2h) after carrageenan injection, in which the edema production is mediated by histamine and serotonin and the late phase (after 2nd h) the vascular permeability is maintained by bradykinin and prostaglandins.[30] These mediators contribute in the inflammatory response and induce pain. Result showed that the second phase of carrageenan-induced edema is sensitive to clinically used anti-inflammatory drugs and commonly employed to assess the antiphlogistic effect of the natural products.[31] Similarly non-steroidal anti-inflammatory drug (NSAID) diclofenac sodium produced significant ($p<0.05$) anti-edematous effect which is consistent with the previous reports.[32,33] Several studies have revealed the inhibitory effects of plant extracts and NSAIDs in similar animal models of pain and inflammation.[34-36] Diclofenac and aspirin suppress inflammation and pain by inhibiting prostaglandin synthesis via inhibition of cyclooxygenase in arachidonic acid pathways.[32]

In the present study, results depicted in Table 4 revealed that, the plant extract of *A. herba-alba*, *E. spinosus* and *A. fragrantissima* exhibited potential inhibitory action on exudate formation. The moderate anti-inflammatory

activity induced by the extracts of *E.hussonii* and *R. epapposum*. Turpentine oil can be used as an irritant. Therefore, turpentine oil-induced granuloma pouch offer a model for exudative type of inflammation which is widely used model for chronic inflammation occurred by means of development of proliferated cells in the form of granuloma. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation.[37] Kinin were the main mediator of granuloma, as it both vasodilate and increase vascular permeability in the early stages of inflammation.[38] Keeping all these in view it may be said that the tested plant extracts may possess anti-kinin like activity. The test plant extracts inhibit the granuloma formation by preventing granulocyte infiltration, generation of collagen fibers, fibroblasts and suppressing mucopolysaccharides.[39] A collective interpretation of the anti-inflammatory data of the test plant extracts (Tables 2-4) revealed that *A. fragrantissima*, *A. herba-alba* and *E. spinosus* demonstrated pronounced activities in the three animal models used in this study and the effect was equal in strength to that of the diclofenac sodium. However, *R. epapposum* and *E.hussonii* induced a moderate activity.

The tested plant extracts found to possess high concentrations of tannins, polyphenols and flavonoids (Table 1). These phytoconstituents could be responsible for anti-inflammatory activity. Flavonoids were known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce significant anti-inflammatory effect due to inhibition of chemical mediators of inflammation.[40] COX-1 and COX-2 catalyze the biosynthesis of prostaglandin H2 from the arachidonic acid substrate. The inhibition of COX-1 results in some undesirable side-effects, whereas COX-2 inhibition provides therapeutic effects in pain, inflammation, cancer, glaucoma, Alzheimer's and Parkinson disease.[41] Therefore, It is speculated to examine the COX-1 and COX-2 inhibitory activity of the studied extracts on purified enzymes as a mechanism of its topical anti-inflammatory action. It was observed that the extracts showed more pronounced COX-1 inhibition as compared to COX-2 (Table 5), which is comparable to diclofenac sodium (non selective COX-2) and lesser than celecocib (selective COX-2). The possible reason might be the different contents and sorts of bioactive compounds, including phenolics, flavonoids and other compounds responsible for the activity. In a similar study, phenolic compounds were shown potent anti-inflammatory activity.[42] Many polyphenolics, tannins and flavonoids were found to inhibit COX-1 and COX-2.[43] The anti-inflammatory effect of extracts may be due to the presence of flavonoids and tannins. It became clear that the mechanism of anti-inflammatory action is assumed to be mediated through the inhibition of COX-1 and COX-2. The study also signifies that phytoconstituents (total phenols and flavonoids) could be responsible, at least in part, for its anti-inflammatory and COX inhibitory activity.

Table. 1: Quantitative Phytochemical Analysis of the Tested Plants.

Plant name	Quantitative analysis (mg/g of methanolic residue)			
	T. Phenolics	Tannins	Non tannins	T. flavonoids
R. epapposum	24.26	-------	24.26	30.2
A. fragrantissima	55.57	36.62	18.95	80.4
A. herba-alba	94.19	71.60	22.59	99.7
E. spinosus	66.54	20.28	46.26	44.6
E. hussonii	46.86	22.90	23.96	23.8

Rhanterium epapposum Oliv. = *R.epapposum*, *Achillea fragrantissima* (Forssk.) Sch.Bip = A.*fragrantissima*, *Artemisia herba-alba* Asso. = A.*herba-alba*, *Echinops spinosus* L. = *E. spinosus*, *Echinops hussonii* Boiss.= *E.hussonii*.

Table. 2: Anti-inflammatory Activity of Some Methanol Extracts of *Asteraceae* family (Acute Inflammatory Model).

Treatment	Volume of edema (mL)			
	0	1h	2h	4h
Control	1.15±0.21	2.67±0.20	3.67±0.18	3.78±0.30
RE (200 mg/kg)	1.04±0.21	1.77±0.11 (51)*	2.92±0.17 (25)*	2.94±0.20 (27)*
RE (400 mg/kg)	1.16±0.11	1.84±0.11 (55)*	2.88±0.30 (31)*	2.73±0.15 (40)*
AF (200 mg/kg)	1.08±0.12	1.63±0.10 (63)*	2.30±0.21 (51)*	2.10±0.20 (61)*
AF (400 mg/kg)	1.20±0.20	1.68±0.14 (68)*	2.10±0.15 (60)*	2.03±0.14 (68)*
AH (200 mg/kg)	1.13±0.20	1.74±0.10 (59)*	2.32±0.30 (52)*	2.59±0.20 (44)*
AH (400 mg/kg)	1.05±0.13	1.54±0.20 (67)*	2.02±0.16 (61)*	2.51±0.14 (56)*
ES (200 mg/kg)	1.20±0.30	2.19±0.11 (34)*	2.42±0.15 (51)*	2.94±0.30 (50)*
ES (400 mg/kg)	1.01±0.20	1.85±0.11 (44)*	1.94±0.12 (63)*	1.89±0.12 (66)*
EH (200 mg/kg)	1.23±0.10	2.03±0.11 (47)*	2.33±0.14 (56)*	2.88±0.30 (37)*
EH (400 mg/kg)	1.11±0.11	1.79±0.20 (55)*	2.10±0.20 (60)*	2.55±0.12 (45)*
DE (20 mg/kg)	1.16±0.20	1.62±0.12 (69)*	2.05±0.13 (64)*	1.95±0.20 (69)*

*Significantly different compared to corresponding control $p \leq 0.05$.

Number of animals = 6. Values are expressed as Mean ±SEM. *RE, R. epapposum*; AF, *A. fragrantissima*; AH, *A. herba-alba*; ES, *E. spinosus*; EH, *E. hussonii*; DE, Diclofenac Na.

Table. 3: Anti-inflammatory activity of some methanol extracts of *Asteraceae* family (Sub-acute inflammatory model).

Treatment	Volume of edema (mL)		
	0 day	1st day	8th day
Control	1.15±0.21	3.78±0.30	6.20±0.30
RE (200 mg/kg)	1.04±0.21	2.94±0.20 (27)*	4.12±0.15 (39) *
RE (400 mg/kg)	1.16±0.11	2.73±0.15 (40)*	3.82±0.20 (47) *
AF (200 mg/kg)	1.08±0.12	2.10±0.20 (61)*	4.01±0.14 (41) *
AF (400 mg/kg)	1.20±0.20	2.03±0.14 (68)*	3.75±0.20 (49) *
AH (200 mg/kg)	1.13±0.20	2.59±0.20 (44)*	3.65±0.14 (50) *
AH (400 mg/kg)	1.05±0.13	2.51±0.14 (56)*	3.13±0.20 (58) *
ES (200 mg/kg)	1.20±0.30	2.94±0.30 (50)*	3.98±0.12 (44) *
ES (400 mg/kg)	1.01±0.20	1.89±0.12 (66)*	3.36±0.30 (51) *
EH (200 mg/kg)	1.23±0.10	2.88±0.30 (37)*	4.80±0.12 (29) *
EH (400 mg/kg)	1.11±0.11	2.55±0.12 (45)*	4.15±0.30 (39) *
DE (20 mg/kg)	1.16±0.20	1.95±0.20 (69)*	3.35±0.20 (59) *

*Significantly different compared to corresponding control $p \leq 0.05$. Number of animals = 6. *RE, R. epapposum*; AF, *A. fragrantissima*; AH, *A. herba-alba*; ES, *E. spinosus*; EH, *E. hussonii*; DE, Diclofenac Na.

Table 4: Anti-inflammatory Activity of Some Methanol Extracts of *Asteraceae* Family in the Turpentine oil – induced Granuloma Pouch Model.

Treatment	Volume of exudates	% of inhibition
Control	2.17±0.21	----------
RE (200 mg/kg)	1.78±0.20*	17.97
RE (400 mg/kg)	1.70±0.30*	21.65
AF (200 mg/kg)	1.51±0.20*	30.41
AF (400 mg/kg)	1.35±0.12*	37.78
AH (200 mg/kg)	1.47±0.13*	32.25
AH (400 mg/kg)	1.32±0.20*	39.17
ES (200 mg/kg)	1.62±0.11*	25.35
ES (400 mg/kg)	1.42±0.21*	34.56
EH (200 mg/kg)	1.80±0.11*	17.05
EH (400 mg/kg)	1.73±0.10*	20.27
DE (20 mg/kg)	1.32±0.20*	39.17

*Significantly different compared to corresponding control $p \leq 0.05$. Number of animals = 6. *RE, R. epapposum*; AF, *A. fragrantissima*; AH, *A. herba-alba*; ES, *E. spinosus*; EH, *E. hussonii*; DE, Diclofenac Na.

Table. 5: *In vitro* COX-1 and COX-2 enzyme inhibition assay data of some Methanol extracts of *Asteraceae* family.

Treatment	% of inhibition		Selectivity
	COX-1	COX-2	COX-2/COX-1
Control	--	---	--
RE (50 µg/ml)	45	49	1.08
AF (50 µg/ml)	32	78	2.43
AH (50 µg/ml)	35	87	2.48
ES (50 µg/ml)	34	65	1.91
EH (50 µg/ml)	43	59	1.37
Diclofenac Na (20µg/ml)	23	56	2.43
Celecoxib (20µg/ml)	8	94	11.75

RE, R. epapposum; AF, A. fragrantissima; AH, A. herba-alba; ES, E. spinosus; EH, E. hussonii;

4. CONCLUSION

Methanolic extracts of *A. fragrantissima*, *A. herba-alba* and *E. spinosus* possesses potential anti-inflammatory activity, which was comparable to diclofenac sodium. However, *R. epapposum* and *E.hussonii* - induced a moderate activity. The mechanism of anti-inflammatory

action was assumed to be mediated through the inhibition of COX-1 and COX-2. It is recommended that the current study verified the traditional use of these plant extracts for the treatment of rheumatism and other inflammatory disorders. This study discovers the possible use of the methanolic extracts of *A. fragrantissima*, *A. herba-alba* and *E. spinosus* in the treatment of inflammatory disorders. This study will help the researcher to uncover and purify the active ingredients of these extracts to become available commercially.

REFERENCES

1. Al-Saeed, A., Gastrointestinal and cardiovascular risk of nonsteroidal anti-inflammatory drugs. Oman Med. J., 2011; 26: 385-391.
2. Yuan, Y., I.T. Padol and R.H. Hunt, Peptic ulcer disease today. Nat. Clin. Pract. Gastroenterol. Hepatol, 2006; 3: 80-89.
3. Verpoorte, R., 1998. Exploration of nature's chemodiversity: The role of secondary metabolites as leads in drug development. Drug Disc. Today 3: 232-238.
4. Vane, J. and R. Booting, Inflammation and the mechanism of anti-inflammatory drugs. J. Feder. Amer. Soc. Exp. Biol. 1987; 1: 89-96.
5. Jonathan, I.A., E.A. David, G. Igelige and A. Adebiyi, A Review on the phytoconstituents and related medicinal properties of plants in the Asteraceae Family. IOSR J. Appl. Chem, 2014; 7(8): 1-8.
6. Al-Harbi, K.B. and I.M. El-Ashmawy, The Antidiarrheal activity and phytoconstituents of some methanol extracts from *Asteraceae* family. Merit Res. J. Med. Medical Sci., 2015; 3(8): 347-352.
7. Aldoweriej, A.M., K.B. Al-Harbi, E.M.A. Saeed, and I.M. El-Ashmawy, Antimicrobial activity of various extracts from some plants native to Alqassim Region, Saudi Arabia. J. of Food, Agriculture & Environ, 2016; 14(1): 14-19.
8. Al-Harbi, K.B., I.M. El-Ashmawy and N.A. Al-Wabel, a. Antidiarrheal activity of the methanol extract of some plants native to Al-Qassim Region, Saudi Arabia. J. Food, Agricul. Environ, 2016; 14(2): 14-19.
9. Al-Harbi, K.B., E.A. El-Tigani-Asil, A.F. Ahmed, I.M. El-Ashmawy and N.A. Al-Wabel, b. Wound healing potential of methanolic extracts of some plants native to Al-Qassim Region, Saudi Arabia. J. Food, Agricult. Environ, 2016; 14(3&4): 18-22.
10. El-Ashmawy, I.M., N.A. Al-Wabel and Bayad, A.E., *Achillea fragrantissima*, rich in flavonoids and tannins, potentiates the activity of diminazine aceturate against *Trypanosoma evansi* in rats. Asian Pacific J. Trop. Med., 2016; 9(3): 228- 234.
11. Al-Farhan, A.H., M.M. Al-Farraj and A.S. Hajar, Wild Edible Plants in Saudi Arabia. Arab Gulf J. Scient. Res., 1992; 10(1): 91-108.
12. Shama, I.Y., I.Y.A. Shama and S.E.I. Adam, Comparative toxicity of Trichodesma africanum and Rhanterium epapposum aerial parts aqueous and methanolic extracts on Wistar rats. J. Pharmacol. Toxicol, 2012; 7: 128-138.
13. Al-Yahya, M.A., I.A. Al-Meshal, J.S. Mossa, A.A. Al-Badr and M. Tariq, Saudi plants, a phytochemical and biological approach. General Directorate of Research Grants Programs. KACST, Riyadh, Saudi Arabia, 1990; 75-80.
14. Shahat, A.A., A.Y. Ibrahim and M.S. Elsaid, Polyphenolic content and antioxidant activity of some wild Saudi Arabian Asteraseae plants. Asian Pac. J. Trop. Med., 2004; 7: 545-551.
15. Elmann, A., S. Mordechay, H. Erlank, A.Telerman, M.Rindner and R.Ofir, Anti-neuroinflammatory effects of the extract of Achillea fragrantissima. BMC Complemen. Altern. Med., 2011; 11: 98-105.
16. Singleton, V. and A. Rossi, Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagent, Am. J. Enol. Vitricult, 1965; 16: 144-158.
17. Osoro, K., A. Mateos-Sanz, P. Frutos, U. Garcı´a, L.M. Ortega-Mora, L.M. Ferreira, R. Celaya and I. Ferre, Anthelmintic and nutritional effects of heather supplementation on Cashmere goats grazing perennial ryegrass-white clover pastures. J. Animal Sci., 2007; 85: 861–870.
18. Zhishen, J., T. Mengcheng and W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 1999; 64: 555-559.
19. Winter, C.A., E.A. Risley and G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 1962; 111: 544-547.
20. Adeyemi, O.O., S.O. Okpo and O.O. Ogunti, Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana mill (lauraceae). Fitoter, 2002; 73(5): 375-380.
21. Robert, A. and J.E. Nezamis, The granuloma pouch as a routine assay for anti- phlogistic compounds. Acta Endocrinol, 1957; 25: 105-107.
22. Shastry, V.M., V.R. Patil and R.Y. Chaudhari, Acute and chronic anti-inflammatory activity of pergularia daemia whole plant in various experimental animal models. Int. J. Pharm. Pharm. Sci., 2011; 3: 241-244.
23. Lee, S.H., M.J.Son, H.K.Ju, C.X.Lin, T.C. Moon, H.G. Choi, J.K. Son and H.W. Chang, Dual inhibition of cyclooxygenases-2 and 5-lipoxygenase by deoxypodophyllotoxin in mouse bone marrow derived mast cells. Biol. Pharm. Bull. 27: 786- 788.
24. Duncan, D.B. 1955. Multiple ranges and multiple F test. Biometrics, 2004; 11: 1-24.
25. SAS. 1987. Statistical Analysis System. Users Guide. Statistics SAS Institute: Cary, North Carolina, USA.
26. Gryglewski, R.J. Molecular mechanism of inflammation. Eur. J. Hematol. Inflamm, 1981; 4: 153-159.

27. Jyothi, M.B., M.K. Jayanthi and R.N. Suresha, Evaluation of anti-inflammatory activity of Aegle marmelos (Bilwa) root. Indian J. Pharmacol, 2011; 43(4): 393-397.
28. DiRosa, M., J.P. Giroud and D.A. Willoughby, Studies of the acute inflammatory response induced in rats its different sites by carrageenan and turpentine. J. Pathol, 1971; 104: 15-29.
29. Vinegar, R., J. F., Truax, J.L. Selph and F.A. Voelker, Pathway of onset development and decay of carrageenan pleurisy in the rat. Fed. Proc., 1982; 41: 2588-2595.
30. Balamurugan, K., G. Sakthidevi and V.R. Mohan, Anti-inflammatory activity of leaf of Melarstoma malabathricum (Melastomataceae).Int. J. Res. Ayur. Pharm, 2012; 3: 801-802.
31. Riaz, N., S.A. Nawaz, N. Mukhtar, A. Malik, N. Afza, S. Ali, S.Ullah, P.Muhammad and M.I. Choudhary, Isolation and enzyme-inhibition studies of the chemical constituents from Ajuga bracteosa. Chem. Biodiver, 2007; 4: 72-83.
32. Vane, J.R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol., 1971; 231: 232-235.
33. Gautam, R., A. Srivastava, S.M. Jachak and A.Saklani, Anti-inflammatory, cyclooxygenase (COX)-2, COX-1 inhibitory and antioxidant effects of Dysophylla stellata Benth. Fitoter, 2010; 81: 45-49.
34. Hernandez, I., L. Marquez, I. Martinez, R. Dieguez, C. Delporte, S. Prieto, J. Molina-Torres and G.Garrido, Anti-inflammatory effects of ethanolic extract and alkamides-derived from Heliopsis longipes roots. J. Ethnopharmacol, 2009; 124(3): 649-652.
35. Boller, S., C. Soldi, M.C. Marques, E.P. Santos, D.A. Cabrini, M.G. Pizzolatti, A.R. Zampronio and M.F. Otuki, Anti-inflammatory effect of crude extract and isolated compounds from Baccharis illinita DC in acute skin inflammation. J. Ethnopharmacol, 2010; 130(20): 1296-1307.
36. Chagas-Paula, D.A., T.B. Oliveira, D.P. Faleiro, R.B. Oliveria and F.B. Costa, Outstanding anti-inflammatory potential of selected Asteraceae Species through the potent dual inhibition of Cyclooxygenase-1 and 5-Lipoxygenase. Planta Med., 2015; 81(14): 1296-1307.
37. Arul, B., R. Kothai, K.Sureshkumar and A.J.M. Christina, Anti-inflammatory activity of coldenia procumbens linn. Pakistan J. of Pharm. Sci., 2005; 18(3): 17-20.
38. Banerjee, T.K., S.S. Mandal, P.C. Das and S. Sikdar, Assessment of the anti- inflammatory effect of Swertia chirata in acute and chronic experimental models in male albino rats. Indian J. Pharmacol, 2005; 32: 21-24.
39. Aruna, D., G.Vinod and N.K. Ajudhia, Evaluation of anti-inflammatory activity of methanolic extract of cassia obtusifolia seeds in Wistar rats. J. Chem. Pharm. Res., 2010; 2(5): 696-700.
40. Singh, S.S., S.C. Pandey, S., Srivastava, V.S. Gupta, B. Patro and A.C. Ghosh, Chemistry and medicinal properties of Salvia moorcroftiana (Guduchi). Indian J. Pharmacol, 2003; 35(2): 83-91.
41. Blobaum, A.L. and L.J. Marnett, Structural and functional basis of cyclooxygenase inhibition. J. Med. Chem, 2007; 50(7): 1425-1441.
42. Parveen, Z., Y. Deng, M.K. Saeed, R. Dai, W.Ahamad and Y.H. Yu, Anti-inflammatory and analgesic activities of Thesium chinense Turcz extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. Yakugaku Zasshi, 2007; 127: 1275-1279.
43. Beg, S., S. Swain, H. Hasan, M.A. Barkat and M.S. Hussain, Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. Pharmacog. Rev., 2011; 5: 120-137.