



Research Paper Evaluation of Anti-Inflammatory Activity of Cassia Angustifolia Seeds Extract in Rat

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 06 Dec 2023	<p>The present study was designed to perform evaluate antiinflammatory activity of seeds of Cassia angustifolia. Seed of Cassia angustifolia was extracted using methanol as solvent by soxhlet apparatus. The evaluation of antiinflammatory activity was done using carrageenan induced rat paw edema assay. The work entitled evaluation of antiinflammatory activity of seeds of Cassia angustifolia was to determine the efficacy and safety in experimental animals. Both aqueous and methanolic extract of seeds of Cassia angustifolia have shown significant reduction in inflammation in proliferative phase as indicated by decreased granuloma formation in the cotton pellet induced granuloma model in rats. The aqueous and alcoholic extract of seeds of Cassia angustifolia has shown significant decrease in preventing generation of collagen fibres, fibroblast and suppressing mucopolysaccharides.</p>
CC License CC-BY-NC-SA 4.0	<p>Keywords: Antiinflammatory, Cassia Angustifolia, Carrageenan, Paw Edema, Petroleum Ether, Aqueous, Methanolic Extract</p>

1. Introduction

The inflammation is a physiological response of a body to stimuli including infections and tissue injury. However, excessive or persistent inflammation can cause a variety of pathological conditions, such as bacterial sepsis, rheumatoid arthritis and skin inflammation (Dinarello, 1997 Palladino et al, 2003). Inflammation is the body's immediate response to damage its tissues and cells by pathogens, noxious substances such as chemicals or physical agents (Weiss, 2008). Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury (Vodovotz et al 2008). However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease. Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor to diseases such as cancer, diabetes and cardiovascular disease (Lucas et al, 2006). High levels of inflammatory cytokines and reactive oxygen species are proposed to contribute pathophysiological mechanisms associated with various inflammatory skin disorders (Trouba et al., 2002). The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury; in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences (Goodman and Gillman, 2006). Cassia angustifolia is a medicinally important plant, its leaves and seeds are given in vomiting, stomachache and headache (Wealth of India, 1992) and roots are used as laxative (R.Zafar, Medicinal plants of India, 1994) and seeds are used in acute inflammation of eye. (Herbs, Species, and Medicinal Plants, 2002). however, the seeds of plant have not been screened for its anti-inflammatory activity so far and phytochemical evaluation of the seeds of the plant shows the presence of phytoconstituents which are found to be responsible for anti-inflammatory activity. The aim of the present study is to investigate the anti-inflammatory activity of seeds of Cassia angustifolia.

2. Materials And Methods

Animals

Wistar albino rats of either sex weighing 180-200 g were procured from Animal house, I.E.S Institute of Pharmacy, Bhopal. The animals were kept in polypropylene cages (3 rats in each cage) at an ambient temperature $25\pm 2^{\circ}\text{C}$ and relative humidity 55-65% respectively. The 12 hr light and dark schedule was maintained. The rats fed with commercially available normal chow diet purchased from Bhopal.

Chemicals

Carrageenan, Diclofenac and Thiopentone were purchased from Sigma Chemical Co., St Louis, MO, USA; Novartis, India and NEON Laboratories Ltd, India respectively. All other chemicals and reagents were of analytical grade (AR).

Plant material

The seeds were collected from Nagpur, India and authenticated at Department of Botany Safia College Bhopal M.P.

Acute Toxicity Study (Botham, 2003)

The acute toxicity study was carried out on rats as per OECD 423 Guidelines, 1987 (Organisation for Economic Cooperation and Development).

Methodology: Single dose of test drug (diluted in 1% CMC) were given to (n=3) using oral rat feeding catheter. Then, the behavioral and clinical manifestations, and mortality were observed upto 14 days.

Phytochemical Screening

Preliminary phytochemical screening of the pet.ether, methanolic and aqueous extract revealed the presence of carbohydrates, triterpenoids, steroids and anthraquinone glycosides.

Drugs And Reagents

Diclofenac and carrageenan (sigma chemical co), carrageenan (sigma chemical co), thiopentone (NEON Laboratories Ltd, India) were used in study

Preparation of Extracts

The seed pods were shade dried and pulverized. The coarse powder was extracted with petroleum ether, 90% methanol using soxhlet apparatus to obtain petroleum ether extract and methanolic extract and remaining marc of methanolic extract further extracted with water to prepare aqueous extract and both methanolic and aqueous extract stored in a desicator till further use.

Animals

Wistar rats of both sexes, weighing 180 – 200 g were used for the study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions. The animals were fed with standard diet (Hindustan liver, Mumbai, India) and had free access to clean drinking water.

Anti-Inflammatory Activity

The anti-inflammatory activity of the extract was determined using carrageenan induced rat paw edema assay. The rats were divided into nine groups of six rats each. The control group received 1% (w/v) in CMC p.o. at a dose of 5 ml/kg. The positive control group was treated orally with the standard drug, diclofenac (20 mg/kg). The test groups received the methanolic and aqueous extract in doses of 250 and 500 mg/kg p.o. All the doses were administered 30 min before the induction of edema by administering 0.1 ml of 1% w/v carrageenan in saline in sub plantar region of hind paw of animal. The degree of paw edema of all the groups was measured using a plethysmograph at 30, 60, 120, 180 min and 3, 4, 24 hrs after the administration of carrageenan to each group.

3. Results and Discussion

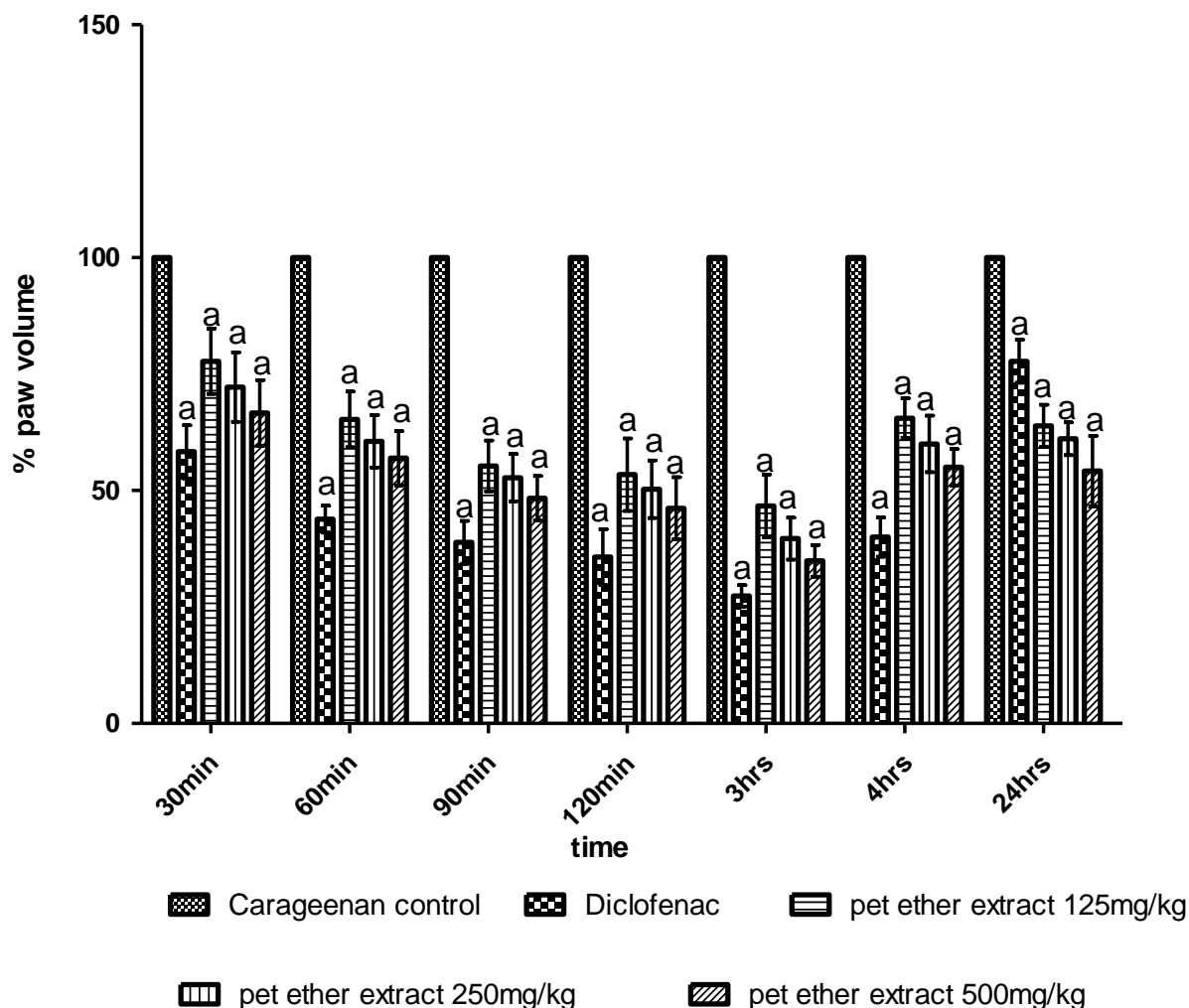


Fig-1. Effect of pet.ether extract of seeds of *Cassia angustifolia* on carrageenan induced paw edema: Results are expressed as Mean \pm SEM. a = $p < 0.05$ statistically significant (n=6).

The pet.ether extract (500mg/kg) showed significant inhibition of ($p < 0.05$) % paw volume at time intervals at 30 min, 60 min, 90min, 120min, 3hrs, 4hrs, 24 hrs (66.66 \pm 7.029, 56.94 \pm 5.84, 48.33 \pm 4.77, 46.19 \pm 6.65, 34.92 \pm 3.41, 55 \pm 3.91, 54.16 \pm 7.58 respectively), Pet ether extract, at the dose of 250 mg/kg has shown significant inhibition ($p < 0.05$) % paw volume at 30 min, 60min, 90min, 120min, 3hrs, 4hrs, 24hrs (72.22 \pm 7.45, 60.55 \pm 5.63, 52.77 \pm 5.05, 39.68 \pm 4.50, 60.22 \pm 6.06, 61.11 \pm 3.51 respectively) compared to carrageenan control group. The Pet. ether extract (125 mg/kg) showed significant ($p < 0.05$) % paw volume at time intervals at 30 min, 60 min, 90min, 120min, 3hrs and 4hrs, 24 hrs (77.77 \pm 7.02, 65.27 \pm 5.98, 55.27 \pm 5.43, 53.41 \pm 5.43 and 46.69 \pm 6.74, 65.55 \pm 4.25, 63.88 \pm 4.52 respectively) in comparison to untreated Control group receiving 1% carrageenan only which is considered as 100% increase in paw volume.

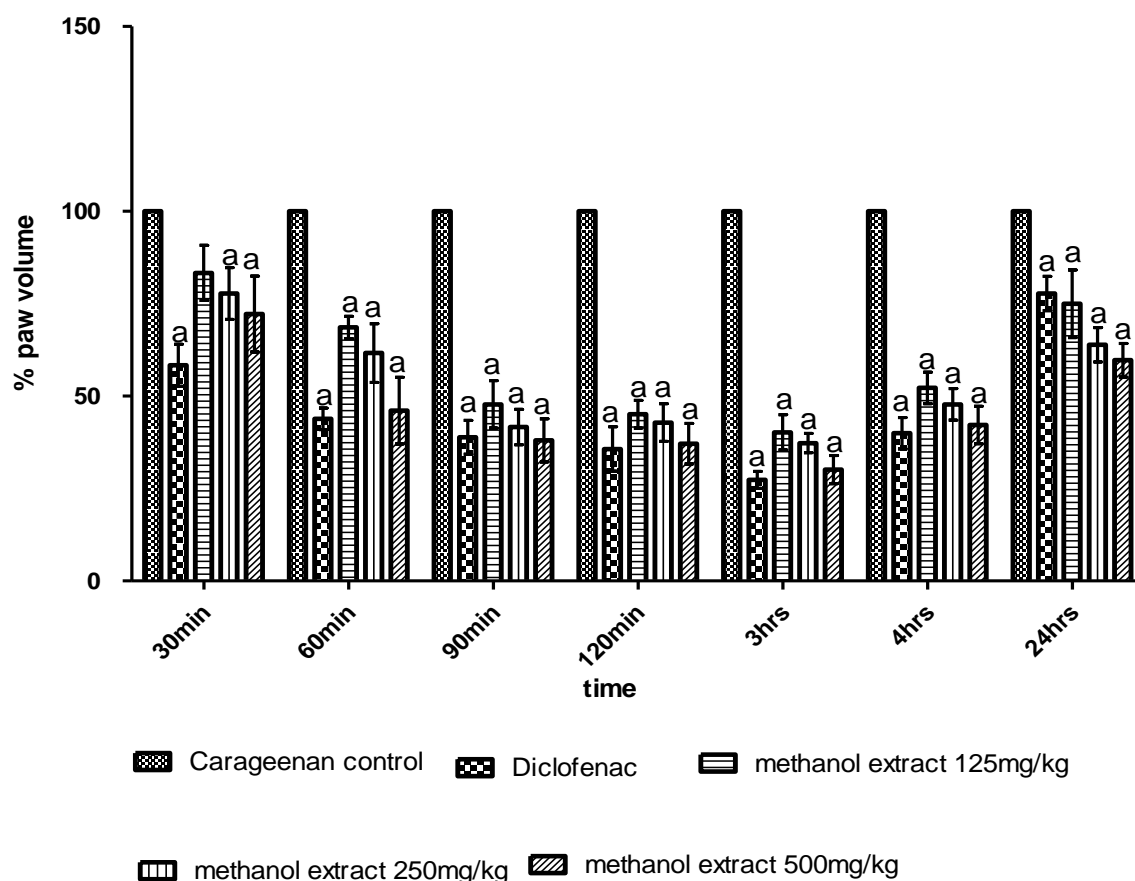


Fig-2. Effect of methanolic extract of seeds of *Cassia angustifolia* drug on carrageenan induced paw edema: Results are expressed as mean \pm SEM. a= $p < 0.05$ statistically significant (n=6).

Methanolic extract (500mg/kg) showed significant inhibition of ($p < 0.05$) % paw volume at 30 min 60 min, 90min, 120min, 3hrs, 4 hrs and 24 hrs (72.22 ± 10.24 , 46.11 ± 9.01 , 38.05 ± 6.18 , 37.14 ± 5.45 , 30.15 ± 9.36 , 42.22 ± 5.06 , and 59.72 ± 4.52 respectively). Methanolic extract at the dose of 250 mg/kg have shown significant inhibition ($p < 0.05$) % paw volume at 30 min, 60min, 90min, 120min, 3hrs, 4hrs, 24hrs (72.22 ± 7.45 , 60.55 ± 5.63 , 52.77 ± 5.05 , 39.68 ± 4.50 , 60.22 ± 6.06 , 61.11 ± 3.51 & 77.77 ± 7.02 , 61.66 ± 7.92 , 41.66 ± 4.77 , 42.85 ± 5.09 , 37.30 ± 2.58 , 47.77 ± 4.27 , 63.88 ± 4.64 respectively) compared to carrageenan control group. The methanolic extract (125mg/kg) showed significant ($p < 0.05$) % paw volume at 60 min, 90min, 120min, 3hrs, 4 hrs and 24 hrs (68.61 ± 3.02 , 47.77 ± 10.52 , 45.10 ± 3.72 , 40.21 ± 4.27 , 75 ± 9.12 , respectively).

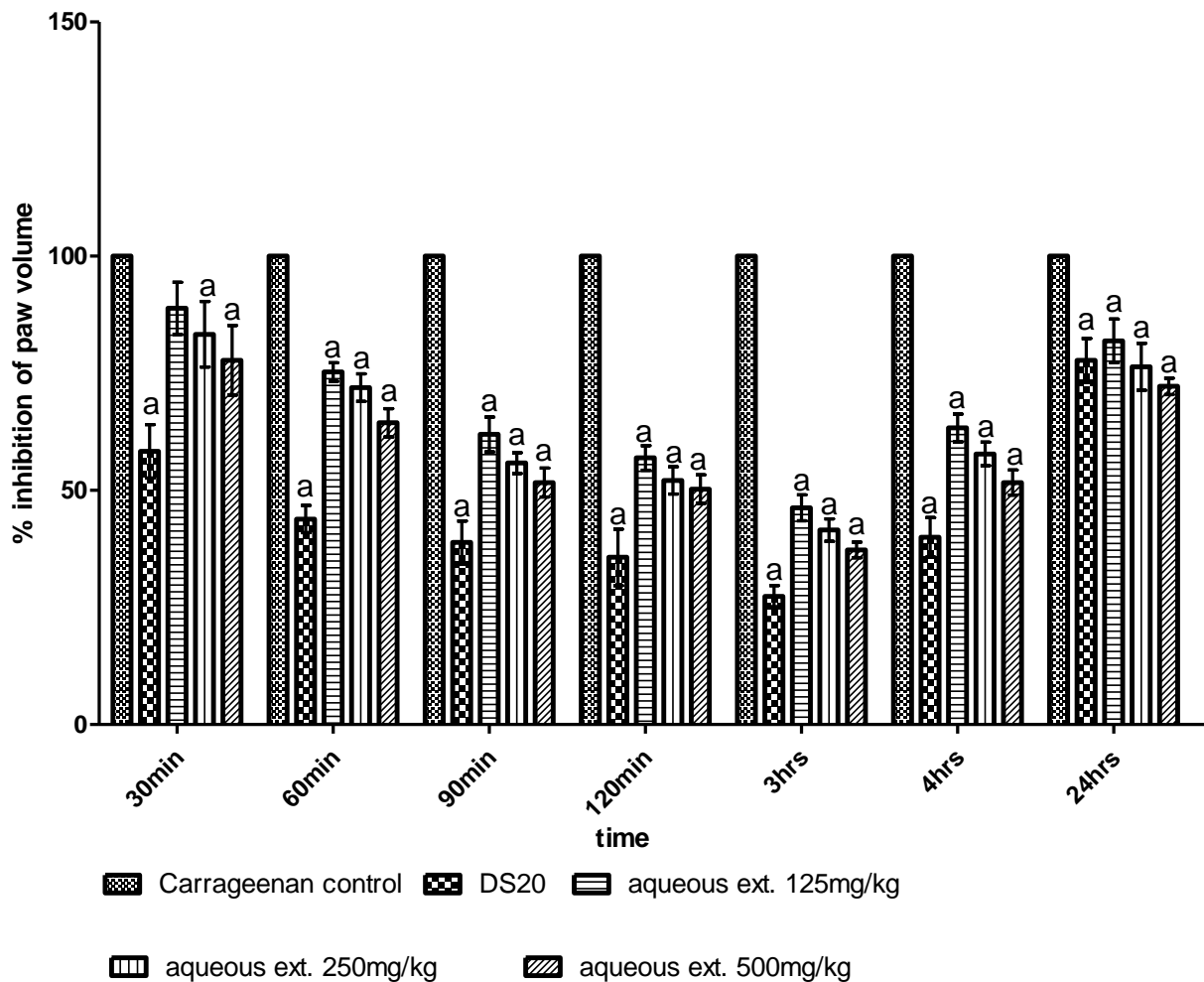


Fig-2. Effect of aqueous extract drug on carrageenan induced paw edema: Results are expressed as Mean ± SEM. a= p<0.05 statistically significant (n=6).

Aqueous extract (500mg/kg) showed significant inhibition of ($p<0.05$) % paw volume at 30 min 60 min, 90min, 120min, 3hrs, 4 hrs and 24 hrs (74.22±10.23, 49.13±9.01, 40.05±6.14, 39.12±5.41, 31.17±9.35, 45.21±5.09, and 60.71±4.55 respectively). Aqueous extract at the dose of 250 mg/kg have shown significant inhibition ($p<0.05$) % paw volume at 30 min, 60min, 90min, 120min, 3hrs, 4hrs, 24hrs (73.21±7.55, 63.51±5.56, 55.74±5.15, 40.65±4.51, 63.25±6.05, 64.16±3.55 & 80.71±6.03, 62.65±8.91, 44.60±5.71, 44.86±4.55, 39.30±3.55, 49.76±4.25, 66.84±4.55 respectively) compared to carrageenan control group. The aqueous extract (125mg/kg) showed significant ($p<0.05$) % paw volume at 60 min, 90min, 120min, 3hrs, 4 hrs and 24 hrs (69.54±3.12, 49.56±12.42, 47.16±4.52, 43.23±4.25, 78±8.13, respectively).

Cotton Pellet Induced Granuloma Model in Rat

In the cotton pellet induced granuloma model, interscapular implantation of sterile cotton pellets have caused significant granuloma tissue formation over the cotton pellet as indicated by elevated cotton weight. Treatment with methanolic extract 500mg/kg showed granuloma tissue formation up to 76.24a ±1.93 whereas standard diclofenac showed 52.49a ±3.71% as compared to control group.

Table 16: Effect of test drug on cotton pellet induced granuloma model in rat:

Group	Granuloma weight	% Granuloma weight
Control	108.27	100±6.29
Diclofenac (15mg/kg)	56.83	52.49 ^a ±3.71
Methanolic extract 500mg/kg	84.87	78.39 ^a ±1.15

n=6, Values were expressed as mean±SEM, a= $p\leq 0.05$ vs control

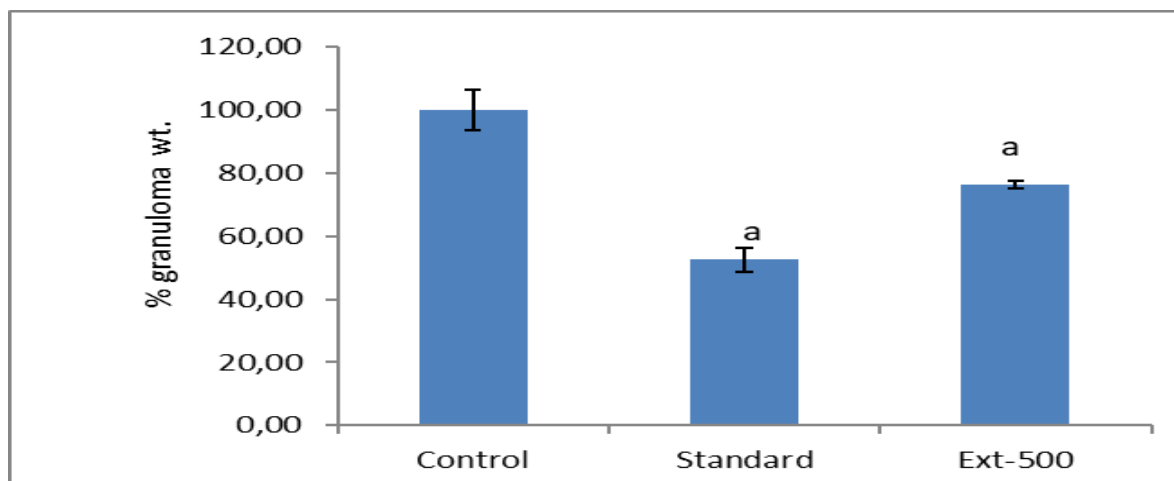


Fig-3. Effect of methanolic extract (500mg/kg) on cotton pellet induce granuloma model : Results are expressed as Mean ± SEM; a= $p \leq 0.05$ statistically significant (n=6).

Discussion

The study demonstrated the protective effect of seeds of *Cassia angustifolia* in acute and chronic inflammation using carrageenan induced paw edema and cotton-pellet induced granuloma models in rats.

In acute toxicity study, the pet. ether, methanolic and aqueous extracts of *Cassia angustifolia* did not show any symptoms of toxicity even at highest dose of 2000mg/kg.

The carrageenan induced rat paw edema test is highly sensitive to nonsteroidal anti-inflammatory drug like Diclofenac has been accepted for investigating or evaluating new drug therapies for inflammatory pathological condition.

Carrageenan reported to cause rat paw edema which is a biphasic event. The initial phase is attributed to the release of histamine and serotonin causing vasodilation and increased capillary permeability; the second phase is due to release of bradykinin, prostaglandins, protease and lysosomal enzymes which regulate the process of adhesion of molecules (ICAM, VCAM) (Naudé et al, 2010) and the process of cell migration, activation and degranulation (Vinegar et al 1969; Crunkhon et al, 1971; Shekar et al, 2009). In general, development of edema induced by carrageenan is correlated with the early exudative and proliferative stages of inflammation. Subcutaneous injection of carrageenan into the rat paw produces plasma and fluid accumulation and plasma protein exudation along with neutrophil extravasation (Chatpaliwar et al, 2002). The early phase of inflammation begins immediately after injection of carrageenan and extends upto 6 hours and the late phase remains upto 24 hours (Guay et al, 2004).

The pet. ether extract of seeds of *Cassia angustifolia* at the doses of 125, 250 and 500mg/kg showed significant ($p < 0.05$) prevention of edema formation from 30 mins to 24 hrs. dose dependently in comparison to their respective untreated control group. The activity was maximum upto 3 hrs.

The methanolic extract at 250 and 500 mg/kg showed significant ($p < 0.05$) prevention in paw edema from 30 min. to 24 hrs. whereas, at 125 mg/kg it showed marked anti-inflammatory effects from 60 min. to 24 hrs. as compared to their respective untreated control rats.

The aqueous extract at the doses of 250 and 500 mg/kg showed marked inhibition of edema from 30 min. to 24 hrs. dose dependently as compared to untreated control rats receiving carrageenan only. Further, the aqueous extract at 125 mg/kg also showed marked anti-inflammatory effects from 60 min. to 24 hrs. as compared to their respective untreated control rats. The aqueous extract showed maximum effect upto 3hrs.

The results obtained from acute anti-inflammatory study revealed that the methanolic extract is having better efficacy in amelioration of carrageenan induced paw edema in all the stages of acute inflammation in comparison to pet. ether and aqueous extracts. Chronic inflammation occurred by means of development of proliferated cells existed in the form of granuloma. Non-steroidal anti-inflammatory drugs like diclofenac inhibit the granulocyte infiltration, preventing generation of collagen fibres, fibroblast and suppressing mucopolysaccharides (Rajavel et al, 2009).

This cotton pellet granuloma model is indicative of proliferative phase of inflammation involving macrophages, neutrophils, fibroblast cells and collagen formation resulting granuloma formation (Kavimani et al, 1999; Balasubramanian et al, 2005).

The methanolic extract of the seeds at dose of 500mg/kg showed marked suppression of proliferative phase as indicated by decreased granuloma formation in the cotton pellet induced granuloma model in rats.

4. Conclusion

In present study, the phytochemical screenings revealed the presence of anthraquinone glycosides, and steroids in pet. ether extract; tri-terpenoid glycosides, carbohydrates in methanolic extract; and carbohydrates, glycosides in aqueous extract. Triterpenoid glycosides like boswellic acids, triterpenic acids have already been reported to have anti-inflammatory effect (Shah et al, 2006; Zhang et al, 2006; Dell'Acquaa et al, 2010; S. Mohammad Abu Darwish et al, 2008). Thus, the marked anti-inflammatory activity exhibited by methanol extract may be due to the presence of triterpenoid glycosides and carbohydrates. Sterols like stigmesterols, β -sitosterols, and brassicasterols showed marked anti-inflammatory effect against acute and chronic inflammations (García et al 1999; Mundkinajeddu et al 2000; Shah et al 2006). Hence, in present study, the pet ether extract showed the anti-inflammatory activity may be due to steroidal phytoconstituents. Carbohydrates and glycosides are also reported to have anti-inflammatory activity and these phytoconstituents may contribute for the antiinflammatory activity of aqueous extract used in present study.

References:

- Balasubramanian, A., Ramalingam, K.K., kumar, S., Christina. A., (2005): Anti-inflammatory Activity of *Morus indica* Linn. Iranian Journal of Pharmacology & Therapeutics., 4: 13-15.
- Botham, P.A., (2003): Acute systemic toxicity—prospects for tiered testing strategies. Toxicology in Vitro., 18 : 227–230.
- Chatpaliwar, V.A., Johrapurkar, A.A., Wanjari, M.M., Chakraborty, R.R., Kharkar, V.T., (2002): Anti-inflammatory activity of *Martynia diandra* glox. Indian Drugs., 39: 543- 545.
- Crunkhon, P., Meacock, S., (1971): Mediators of the inflammation induced in the rat paw by carrageenan. British Journal of Pharmacology., 42 : 392-402.
- Dell'Acquaa S., Castagliuolo I , Brunb P., Ditadib F. , Palùb G., Innocentia G.,(2010): Triterpene glycosides with in vitro anti-inflammatory activity from *Cyclamen repandum* tubers. Carbohydrate Research., 345(5): 709-714.
- Dinareello CA., (1997): Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. Chest 112: 321–329
- García, M. D., Sáenz, M. T., Gómez, M. A., Fernández, M. A.,(1999): Topical antiinflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. Phytotherapy Research.,13 (1) : 78 – 80.
- Goodman and Gilman's, (2006): The Pharmacological Basis of Therapeutics. McGrawhill, 11thEd., pp. 687-696.
- Guay, J., Bateman, K., Gordon, R., Mancini, J., Riendeau, D., (2004): Carrageenan-induced Paw Edema in Rat Elicits a Predominant Prostaglandin E2 (PGE2) Response in the Central Nervous System Associated with the Induction of Microsomal PGE2 Synthase-1. The journal of biological chemistry., 279(23): 24866–24872.
- Herbs, Species, and Medicinal Plants(2002):CBS Publishers and distributors,New Delhi.,pp.104
- Kavimani, S., Llango, R., Loganathan, C., Karpagam, S., Jaykar, B., (1999): Anti-inflammatory activity of benidipine hydrochloride. Indian Drugs., 36: 147-149.
- Lucas, S.M., Rothwell, N.J., Gibson, R.M., (2006) : The role of inflammation in CNS injury and disease. British Journal of Pharmacology., 147: S232–S240.
- Mundkinajeddu, Deepak., Handa, Sukhdev, Swami., (2000) : Antiinflammatory activity and chemical composition of extracts of *Verbena officinalis*. Phytotherapy Research.,14 (6): 463- 465
- Naudé, P.J.W., Cromarty, A.D.E., Constance, Rensburg, J., van., (2010): Potassium humate inhibits carrageenan-induced paw oedema and a graft-versus-host reaction in rats. Inflammapharmacology., 18(1): 33-39.
- Palladino, M. A., Bahjat, F. R., Theodorakis, E. A., Moldawe, L. L., (2003). Anti-TNF-alpha therapies: the next generation. Natl. Rev. Drug Discov., 2 : 736–746.
- R.Zafar, (1994): Medicinal plants of India, 1st Edn.,CBS publishers and distributors,New Delhi,India,pp.49-61
- Rajavell, R., Thangavel S., Murugesan J., Malliga, P., (2009): Evaluation of analgesic and anti-inflammatory activities of *Oscillatoria willei* in experimental animal models. Journal of Medicinal Plants Research., 3(7) : 533-537.
- Shah, Biren., Nayak, B.S., Seth, A.K., Jalalpure, S.S., Patel, K.N., Patel, M.A., Mishra, A.D., (2006): Search for medicinal plants as source of anti-inflammatory and anti-arthritic agents-A review. Phcog Mag., 2(6): 77-86.
- Shekar, D. Sheshadri., V. C., Ramakrishnan, G., Vivek, B.,(2009): Anti-Inflammatory Activity of Ethanolic and Acetone Extracts of *Bauhinia Variegata*. Research J. Pharm. and Tech.,2(2):409-411.
- Trouba, K.J., Hamadeh, H.K., Amin, R.P., Germolec, D.R.,(2002). Oxidative stress and its role in skin disease. Antioxid. Redox Signal. 4, 665–673.

- Vinegar, R., Schreiber, W., Hugo, R.,(1969): Biphasic development of carrageenan oedema in rats. *Journal of Pharmacological Experimental Therapeutics.*, 66: 96-103.
- Vodovotz, Y., Csete, M., Bartels, J., Chang, S., An, G., (2008). Translational systems biology of inflammation. *PloS Computational Biology.*
- Wealth of india(1992):Publication and information directorate council of scientific and industrial research ,New Delhi, 3, pp. 349.
- Zhang, D., Marconi, A., Xu, L., Yang, C., Sun, G., Feng, X.-l., Ling, Chang-q, Qin, W., Uzan, G., Alessio, P., (2006): Tripterine inhibits the expression of adhesion molecules in activated endothelial cells. *Journal of Leukocyte Biology*, 80:309-319.