Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Special Issue-**2** **Year 2023** Page **3570:3579***

Assessment of *In-vivo* Anti-inflammatory Potential of Fruit Extract of Ashwagandha (*Withania somnifera*) Using Carrageenan Induced Paw Edema Rat Model Study

Akhila A[1] and Keshamma E[2*]

[1]Associate Professor, Department of Zoology, Govt. First Grade College, Vijayanagar, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Biochemistry, Maharani Science College for Women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

***Corresponding Author**

Dr. Keshamma E

Associate Professor,

Department of Biochemistry,

Maharani Science College for Women, Maharani Cluster University,

Palace Road, Bengaluru-560 001,

Karnataka, India

Email: keshamma.blr76@gmail.com

Article History

Received: 08 Aug 2023

Revised: 29 Sept 2023

Accepted: 29 Oct 2023

Abstract

Ethnobotanicals are important for pharmacological research and drug development. Indian medicinal plant Ashwagandha (*Withania somnifera*) has been used in the Ayurvedic system of medicine for the treatment various human ailments. Non-steroidal antiinflammatory drugs (NSAIDs) that are mainly used in the treatment of pain and inflammation with side effects like gastrointestinal irritation. Therefore, there is a resurgence to search for alternative anti-inflammatory drugs and medicines from natural sources. Hence, in the current study we aimed to assess the anti-inflammatory potential of Ashwagandha fruits in an *in-vivo* carrageenan induced paw edema rat model study. Fruits of Ashwagandha was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. Anti-inflammatory activity of methanolic fruit extract of Ashwagandha at doses of 150 mg/kg, 300 mg/kg and 600 mg/kg was evaluated in carrageenan induced paw edema Wistar albino rat model test. Inflammation was produced by administering 0.1 ml of 1% carrageenan into sub-plantar surface of rat hind paw to negative control group; 150 mg/kg (Group-I), 300 mg/kg (Group-II), 600 mg/kg (Group-III) methanolic fruit extract Ashwagandha and Aspirin 100 mg/kg (positive control) was administered

intraperitoneally respectively. Results depicted that there was a dose dependent inhibition of hind paw edema volume following treatment with methanolic fruit extract of Ashwagandha. Furthermore, the anti-inflammatory effect of methanolic fruit extract Ashwagandha extract at the dose level of 300 mg/kg was comparable with that of standard drug *viz.* Aspirin. Moreover, maximum inhibition of paw edema volume was observed at 12 h time interval at all the dose levels of methanolic fruit extract Ashwagandha. In conclusion, this preliminary study demonstrated the anti-inflammatory potential of methanolic fruit extract of Ashwagandha. Therefore, Ashwagandha fruit extract could be considered for development of natural anti-inflammatory drugs.

Keywords: Ashwagandha, *Withania somnifera*, Anti-inflammatory, Carrageenan

Introduction

Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds.[1] It is not surprising that from conception to market most compounds face an uphill battle to become an approved drug. For approximately every 5,000 to 10,000 compounds that enter preclinical testing, only one is approved for marketing. Drug research and development is comprehensive, expensive, time-consuming and full of risk. On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs.[2] As we know that anti-inflammatory drugs are useful for many long-standing diseases such as rheumatoid arthritis, osteoarthritis, hence there is need of a drug with minimum side effects and can be useful for acute and chronic diseases.

Withania somnifera, also known as Ashwagandha is a small, woody shrub in the Solanaceae family that grows about two feet in height. It can be found growing in Africa, the Mediterranean and India. An erect, evergreen, tomentose shrub, 30-150 cm high, found throughout the drier parts of India in waste places and on bunds (Figure 1). Roots are stout fleshy, whitish brown; leaves simple ovate, glabrous, those in the floral region smaller and opposite; flowers inconspicuous, greenish or lurid-yellow, in axillary, umbellate cymes; berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow, reniform. The bright red fruit (Figure 1) is harvested in the late fall and seeds are dried for planting in the following spring.[1]

Figure 1: Showing Ashwagandha plant and fruits

Inflammation can be described as a complex body response to injurious stimuli like pathogens, irritants, or damaged cells.[3] Inflammatory responses are generated and maintained through the interactions of inflammatory mediators, such as histamine, kinins, cytokines, eicosanoids, calcitonin gene-related peptide, substance-P, and platelet-activating factor, derived from leukocytes and damaged tissues. The primary manifestations of inflammation are the infiltration of leukocytes and the formation of edema. Edema formation is a consequence of an inflammatory mediator's interaction that promotes vascular permeability and blood flow.[3,4] Various synthetic drugs such as NSAIDs have good efficacy in inflammatory conditions but also exert some deleterious effects including gastrointestinal problems.[5] Hence, herbal remedies have always been important.

Medicinal plants have a significant role in sustaining human health in developing countries due to lack of primary healthcare facilities, poverty, increased demand for inexpensive medicines, and drug-associated fewer side effects.[6] Plants provide a source of bioactive compounds that possess pharmacologically important properties.[7] Indian medicinal plant Ashwagandha has been used in the Ayurvedic system of medicine and has an anti-arthritic, anti-bacterial, anti-oxidant, antidiabetic, anti-tumour, anti-inflammatory activity, immunomodulatory activity, and analgesic activity, anti-sertogenic activity, anabolic activity and anti-stress activity. It also possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties.[8] With this context the present study was conducted with the main the purpose of assessment of *in-vivo* anti-inflammatory activity of Ashwagandha fruits using carrageenan induced paw edema rat model study.

Materials and Methods

Collection of Ashwagandha Fruits

The fruits of Ashwagandha were purchased from the local markets of Vijayanagar located in West-Bengaluru region, Karnataka, India. The locally purchased fruits of Ashwagandha were sprayed with ethanol, and then shade dried at room temperature for 24-48 hrs. The dried fruits of Ashwagandha were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 100 g of dried and finely powdered fruits of Ashwagandha were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of methanol. The extracts were concentrated by distilling the solvent in a rotary flash

evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.[9,10]

Estimation of Total Phenolic Content (TPC)

The TPC was determined by the modified method of Folin-Ciocalteu method as described by Hatami et al.[11] Briefly, 500 µL of different concentrations of extracts was mixed with 0.5 mL of 10-fold diluted Folin-Ciocalteu reagent. After 5 min 0.5 ml of 7.5% Sodium carbonate solution, 4.5 ml of double distilled water were added, vortexed and incubated in a dark place for 120 min, the optical density was measured at 760 nm against a blank. The TPC was calculated on the basis of the calibration curve of gallic acid standards (10 ppm-100 ppm) and expressed as gallic acid equivalents (GAE), in milligrams per gram of the sample (GAE, mg/g sample).

Total Flavonoids

Aluminum chloride colorimetric method was used for total flavonoids determination.[12] The total flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of total flavonoid was expressed in terms of gallic acid equivalents (GAE), in milligrams per gram of the sample (GAE, mg/g sample).

***In-vivo* Anti-inflammatory Activity Assay**

Sample preparation

The extract was mixed with methanol at the final concentration of 2 mg/mL and was used as sample extract for determination of anti-inflammatory activity.

Ethical approval

The study was conducted in compliance with the guidelines laid down by the Institutional Animal Ethics Committee (IAEC) approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental animals

Healthy Wistar albino rats weighing between 150-200 g were used. The rats were maintained at 25ºC with relative humidity of 45 to 50% and under standard environmental conditions with 12:12 h light/dark cycle in polypropylene cages for one week before the experiments. The animals were fed with standard pellet feed and water was given *ad libitum*. The animals were deprived of food for 24 hours before experimentation, but had free access to drinking water. All experiments were performed in the morning.

Assay of Anti-inflammatory Activity

Carrageenan-induced inflammatory model:

Inflammation was produced by administering 0.1 ml of 1% carrageenan into sub-plantar surface of rat hind paw. Albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water.[13] The animals were divided in to five groups as follows;

Study Design

Groups	Treatment	No. of Animals / Group
Negative Control (NC)	Distilled water (10 ml/kg) + Carrageenan (0.1ml of 1% normal saline)	6
Positive Control (PC)	Aspirin (100 mg/kg, i.p.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6
Treatment-I	Methanolic Fruit Extract of Ashwagandha (150 mg/kg, i.p.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6
Treatment-II	Methanolic Fruit Extract of Ashwagandha (300 mg/kg, i.p.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6
Treatment-III	Methanolic Fruit Extract of Ashwagandha (600 mg/kg, i.p.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6

Selected doses of methanolic fruit extract of Ashwagandha was given intraperitonially. One hour after drug treatment all animals were injected with 0.1 ml of 1% carrageenan solution in the sub-plantar aponeurosis of left hind paw and the paw volume was measured plethysmometrically. Recordings were taken up to 12 hours at 1h, 2 h, 4 h, 6 h, and 12 h intervals. The % inhibition in paw volume was calculated by using following formula;[14]

% inhibition in paw volume = $100 \times (1 - Vt/Vc)$

Where,

Vt = mean paw volume in the drug treated group

Vc = mean paw volume in negative control group

Statistical Analysis

The data are expressed as Mean ± standard deviation (SD)

Results and Discussion

Total Phenolic Content (TPC) and Total Flavonoid

The results of TPC and total flavonoid content of methanolic fruit extract of Ashwagandha was represented in Table 1. Results revealed that TPC of methanolic fruit extract of Ashwagandha was found to be highest (129.58 GAE mg/g) as compared to total flavonoid content of methanolic fruit extract of Ashwagandha (28.28 GAE mg/g).

Table 1: Total phenolic content (TPC) and total flavonoids in Ashwagandha fruit extracts

Phytochemicals	Methanolic Fruit Extract of Ashwagandha
Total Phenolic Content (TPC)	129.58 ± 11.48 GAE mg/g

Total Flavonoids	28.28 ± 2.68 GAE mg/g

Values were expressed Mean ± SD; n=3; GAE, Gallic acid equivalents

The results of the anti-inflammatory effect of methanolic fruit extract of Ashwagandha and positive control drug was as represented in Figures 1, 2, 3, 4, and 5. Results depicted that there was a dose dependent inhibition of hind paw edema volume following treatment with methanolic fruit extract of Ashwagandha at the concentrations of 150 mg/kg (Treatment I), 300 mg/kg (Treatment II), and 600 mg/kg (Treatment III) at all the time intervals recorded i.e., 1 h, 2 h, 4 h, 6 h and 12 h. Furthermore, inhibition of hind paw edema volume of methanolic fruit extract of Ashwagandha was comparable with that standard drug i.e., Aspirin.

Figure 1: Effect of methanolic fruit extract of Ashwagandha on inhibition of carrageenan induced hind paw edema in rats at 1 h

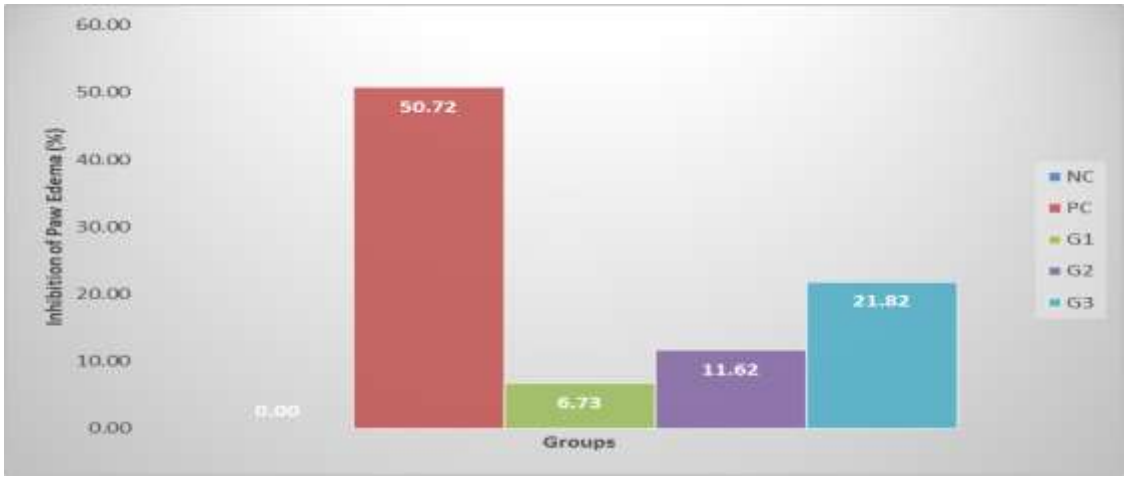


Figure 2: Effect of methanolic fruit extract of Ashwagandha on inhibition of carrageenan induced hind paw edema in rats at 2 h

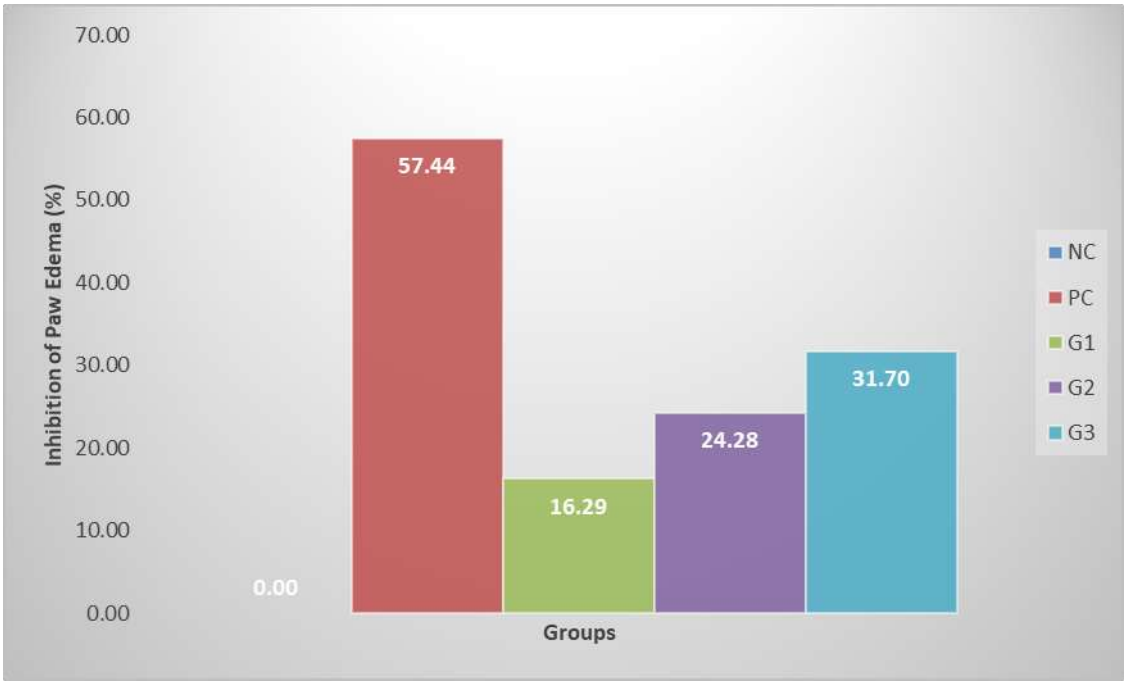


Figure 3: Effect of methanolic fruit extract of Ashwagandha on inhibition of carrageenan induced hind paw edema in rats at 4 h

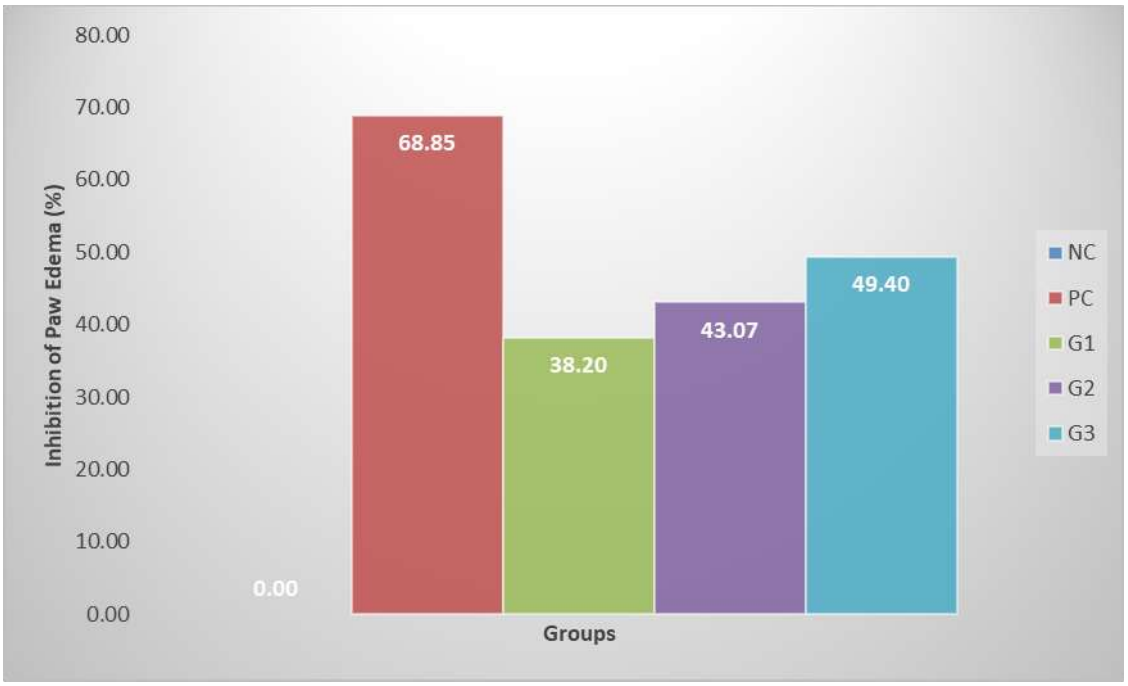


Figure 4: Effect of methanolic fruit extract of Ashwagandha on inhibition of carrageenan induced hind paw edema in rats at 6 h

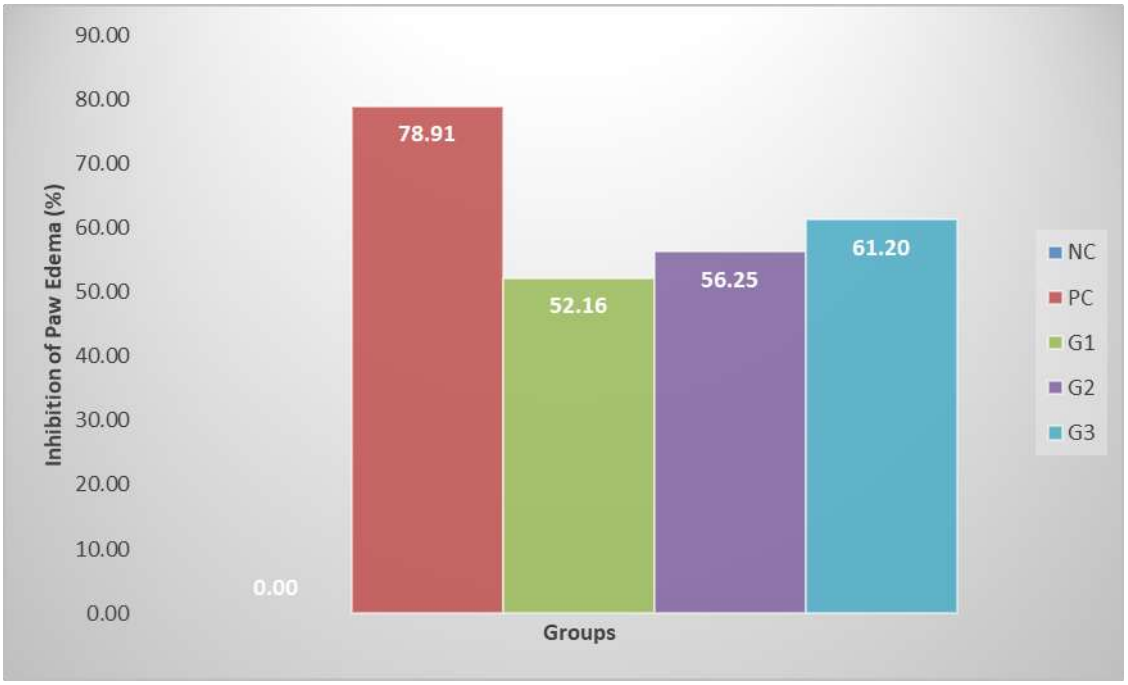
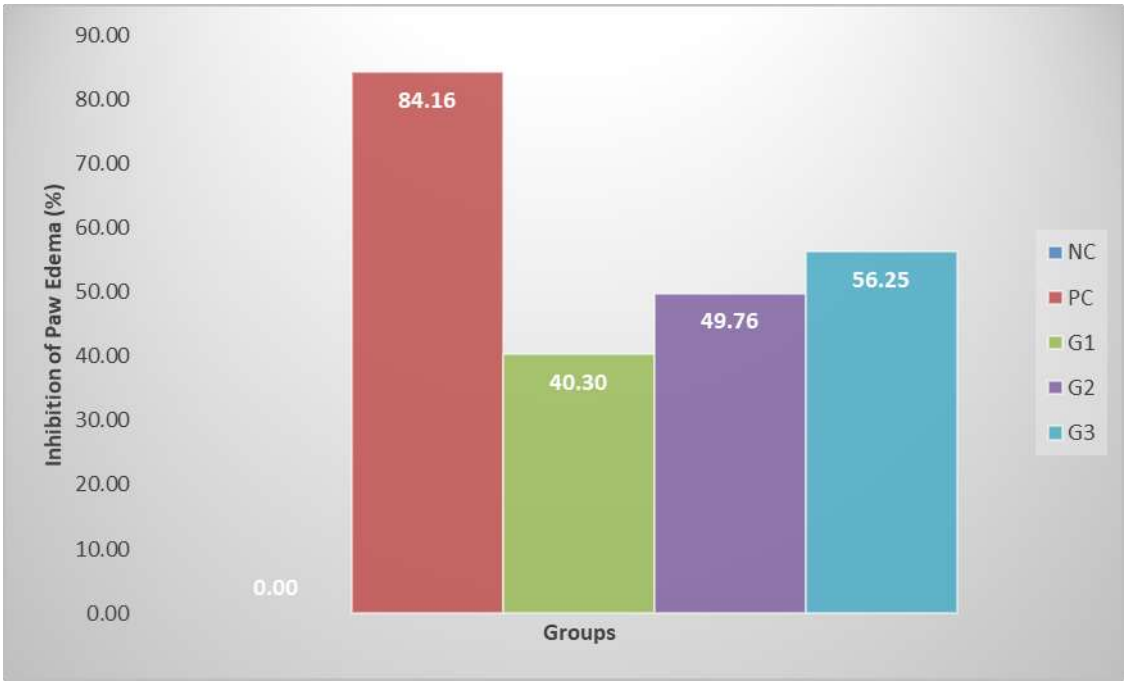


Figure 5: Effect of methanolic fruit extract of Ashwagandha on inhibition of carrageenan induced hind paw edema in rats at 12 h



Pain and edema are the outcome of inflammatory reaction. Pain has been described as nature's early sign of morbidity. It was reported that chemical production of pain usually results in an inflammatory response in biologically active system.[15] In the present study we aimed to assess the *in-vivo* anti-inflammatory activity of methanolic fruit extract of Ashwagandha using carrageenan induced paw edema rat model study. The carrageenan-mediated biphasic inflammatory response is arbitrated by an upsurge of histamine, kinins, and serotonin in the initial phase and release of acute inflammation associating prostaglandins.[16] Carrageenan induced edema model is a better model to assess *in-vivo* anti-inflammatory activity of substances.[17] Therefore, the same method is adopted in our study.

The development of edema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances.[18] Methanolic fruit extract of Ashwagandha test for *in-vivo* anti-inflammatory activity was able to suppress edema and this effect may be due to the inhibitory effects on the release of histamine, 5-hydroxyl tryptamine and kinin like substances which are reported to release from mast cell degradation during the first hour of carrageenan induced artificial paw edema.[19] This decrement of paw volume following treatment methanolic fruit extract of Ashwagandha observed in our study might be due to the presence of phenolic and flavonoid compounds present in the extract; since phenolic and flavonoids compounds are reported to produce anti-inflammatory action by decreasing capillary permeability.[20]

Conclusion

In conclusion, this initial study demonstrated the anti-inflammatory potential of methanolic fruit extract of Ashwagandha (*W. somnifera*). Therefore, Ashwagandha fruits extract could be considered for development of natural anti-inflammatory drugs. However, further studies are warranted to elucidate the exact mechanism of action of anti-inflammatory action of Ashwagandha (*W. somnifera*) extract.

References

1. Li JWH, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science. 2009;325(5937):161-5.
2. Kumari KM, Bairy KL, Shenoy S. Evaluation of antiinflammatory and analgesic activities of alcoholic extract of *Kaempferia galanga* in rats. Indian J Physiol S. Scientific Basis for the Therapeutic Use of *Withania somnifera* (Ashwagandha): A Review. Altern Med Rev. 2000;5(4):334-46.
3. Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol. 2012;4(3):a006049.
4. Nathan C. Points of control in inflammation. Nature. 2002;420(6917):846-52.
5. Tasneem S, Saleem M, Saeed SA. Nonsteroidal anti-inflammatory drugs as potential ecto-nucleotide phosphodiesterase inhibitors. Braz J Pharm Sci. 2020;56:e18271.
6. Hao DC, Xiao PG. Pharmaceutical resource discovery from traditional medicinal plants: Pharmacophylogeny and pharmacophylogenomics. Chin Herb Med. 2020;12(2):104-17.
7. Bahadur S, Khan MS, Shah M, Shuaib M, Ahmad M, Zafar M et al. Traditional usage of medicinal plants among the local communities of Peshawar valley, Pakistan. Acta Ecol Sin. 2020;40(1):1-29.
8. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): a review. Altern Med Rev. 2000;5(4):334-46.
9. Rauf A, Khan A, Uddin N, Akram M, Arfan M, Uddin G et al. Preliminary phytochemical screening, antimicrobial and antioxidant activities of *Euphorbia milii*. Pak J Pharm Sci. 2014;27(4):947-51.
10. Kamurthy H, Dontha S, Rajani K. Phytochemical screening on *Euphorbia milii* red flowers-isolation of terpenoids, flavone and phenols. Am J Ethnomed. 2015; 6:322-32.
11. Hatami T, Emami SA, Miraghaee SS, Mojarrab M. Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of *Artemisia biennis* Willd. Iran J Pharm Res. 2014;13(2):551-9.
12. Ordonez AAL, Gomez JD, Vattuone MA, Lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452-8.
13. Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti-inflammatory activity of *Callistemon lanceolatus* leaf. Asian Pac J Trop Biomed. 2011;1(3):177-81.
14. Benni JM, Jayanthi AR, Suresh RN. Evaluation of the antiinflammatory activity of *Aegle marmelos* (Bilwa) root. Ind J Pharmacol. 2011; 43:393-7.
15. Uthirapathy S, Anwer ET, Ahamad J, Porwal O, Mohammed Ameen MSH. Anti-atherogenic effect of *Terminalia arjuna* through suppressing adipocytokines and cardiac markers, JETIR. 2019;6(5):313-26.
16. Michael SU, Aliyu U, Ibrahim T, Samuel EO, Samuel TM, Bulus A. Evaluation of active phytochemical constituents linked to the analgesic and anti-inflammatory property of *Cassia singueana* Del. root bark. J Med Plants Res. 2019;13(12):288-95.
17. Winter CA, Risley GA, Nass GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 96;12(3):544-7.

18. Vetriselvan S, Subasini U, Velmurugan C. Anti-inflammatory activity of *Cucumis sativus* seed in carrageenan and xylene induced edema model using Albino Wistar rats. Int J Biopharm. 2013;4(1):34-7.
19. Vinegar R, Truax JF, Selph JL, Johnston PR, Venable AL, McKenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed Proc. 1987;46(1):118-26.
20. Subashini U, Mohamed M, Shabi G Krishnamoorthy, Dhevi Ravichandran. Victor G. Rajamanickam, Govindha Pillay Dubey. Analgesic and Anti-arthritic effect of *Corallacarpus Epigaeus*. Clin Biochem (Acta Bioquimica Clinica Latinoamericana. 2011;45(4):749-56.