Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 13 (2024), Article ID 236282, 06 pages
DOI: 10.4303/JDAR/236282

Research Article

Pharmacological Screening of Antiinflammatory Activity of Flemingia chappar

Madduri Padmavathamma*, Shanmugapandian. P

Department of Pharmacy, Prist Deemed University, India

Address Correspondence to Madduri Padmavathamma, E-mail: padmmavathammamm@gmail.com

Received: 31 January 2024; Manuscript No: JDAR-24-124393; **Editor assigned:** 02 February 2024; PreQC No: JDAR-24-124393 (PQ); **Reviewed:** 16 February 2024; QC No: JDAR-24-124393; **Revised:** 21 February 2024; Manuscript No: JDAR-24-124393 (R); **Published:** 28 February 2024; **DOI:** 10.4303/JDAR/236282

Abstract

Pharmacological screening of anti-inflammatory activity of ethanolic extract of from the Whole plant Flemingia chappar (Graham) were analysed in this study. Inflammation is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury, or tumor growth leading to local accumulation of plasma fluid and blood cells.

The plant from the whole plant of Flemingia chappar 2 main types of anti-inflammatory agents, namely, glucocorticosteroids and Nonsteroidal Anti-inflammatory Drugs (NSAIDs). These, analgesic agents which lack gasic lision effects are being searched all over the world as alternatives to NSAIDs and SAIDs. In the current research, an effort was made to study the analgesic and anti-inflammatory of ethanolic extract of whole plant of Flemingia chappar (Graham) by following pharmacological screening methods. Anti-inflammatory activity was done by carragenan–induced rat paw oedema, Histamine induced rat paw edema, dextran induced rat paw edema, as inducing agents. Results of ethanolic extract of whole plant of Flemingia chappar (Graham) were compared with the efficacy of standard anti-inflammatory drugs. These findings justify that from the whole plant Flemingia chappar (Graham) can be a valuable natural anti-inflammatory source which seemed to provide potential Phyto therapeutics against various ailments. Ethanolic extract whole plant of Flemingia chappar ethanolic extract (Graham) was systematically evaluated for its anti-inflammatory potential by following standard pharmacological screening methods. Results suggested that the ethanolic extract of FCE found to possess comparable efficacy with that of standard anti-inflammatory drugs.

Keywords: Flemingia chappar; Anti-inflammatory; Ethanolic extract; Carrageenan induced rat paw

Introduction

Ethnobotanical research done in the last few decades has revealed the anti-inflammatory properties of plants cited in the traditional literature. Many herbal preparations are being prescribed as anti-inflammatory and analgesics in the traditional literature. The search for new anti-inflammatory agents from the huge array of medicinal plant resources is intensifying. This is because such taxa may hold assurance for the discovery of novel therapeutic agents capable of suppressing, reducing, or relieving pain as well as inflammation [1]. Inflammation is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury, or tumor growth leading to local accumulation of plasma fluid and blood cells [2]. Edema formation, leukocyte infiltration, and granuloma formation represent such components of inflammation [3]. There are 2 main types of anti-inflammatory agents, namely, glucocorticoids and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). Due to adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents has not been successful in all the cases. Therefore, analgesic drugs which lack those effects are being searched all over the world as alternatives to NSAIDs and opiates. Drug research and development (R & D) is comprehensive, expensive, time-consuming, and full of risk. It is estimated that a drug from concept to market would take approximately 12 years and capitalizing out-of-pocket costs to the point of marketing approval at a real discount rate of 11% yields a total preapproval cost estimate of US $ 802 million [4]. 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 [5]. The investigation of the efficacy of plant-based drugs used in the traditional medicine has been paid great attention because they are cheap, have little side effects, and according to WHO still about 80% of the world population rely mainly on plant-based drugs [6]. The whole plant of Flemingia chappar (Graham) (Family: Fabaceae), is an erect herb, young shoots are covered with yellow,

soft tomentose hairs. The leaf is opposite, alternate, simple, entire, the flowers are renames, spike, panicle. In the current research, an effort was made to study the anti-inflammatory of ethanolic extract of from the whole plant of Flemingia chappar (Graham) by following pharmacological screening methods.

Materials and Methods

Chemicals and reagents

Petroleum ether, ethanolic extract, carragenan Indomethacin, Mayer's reagent, Dragendorff's, Wagner's, Hager's, Fehling's test, Molish's test Benedict's test and Ferric chloride. All other reagents and chemicals used were of analytical grade.

Plant material entire parts of plant of Flemingia chappar Graham collected duringin February 2016-April 2016 from the Tirumala hills, Andhra Pradesh, India [4,7-9]. The plant was authenticated by Dr. Madhava Chetty, Taxonomist, S.V. University, Tirupati, India Flemingia chappar Graham voucher number 0459. The collected plant was cleaned immediately and shade-dried for a week, powdered mechanically, sieved (10/44) and stored in airtight containers.

Extraction

5000 grams of the whole plant powder was accurately weighed and soaked in various solvents extract to active ingredients using analytical grade solvents starting with Highly non polar petroleum ether (60°C–80°C) to successively increasing the polarity *viz*. ethanolic extract (95%) following soxhlation method. All the extracts were concentrated by using rota–vacuum evaporator (Buchi type, Mumbai, India) until a semisolid extract is obtained, dried at less than 50°C, preserved in air tight containers kept in desiccators prior to its studies.

Phytochemical investigation

Phytochemical investigation of whole extract was carried out for all the extracts, using analytical grade reagents. The respective yields and the phytochemical investigation results were given in Table 1.

Table 1: Results of preliminary phytochemical studies

Constituent	FCP	FCC	FCA	FCEA	FCE
Carbohydrates					
Molish`s test	-	+	++	++	+++
Fehling`s test	-	+	++	++	++
Benedict`stest	-	+	++	++	++
Saponins					
Foam test	+	+	+	+	+++
Flavonoids					
Ferric chloride test	+	+	+	++	++
Shinoda test	+	+	+	+	+
Lead acetate test	+	+	+	+	+
Alkaline reagent test	+	+	+	+	++
Steroids					
Libermann-Burchard test	+	+	+	+	+++
Salkowski test	+	+	+	+	+++
Proteins					
Millon`s test	++	++	++	++	++
Biuret test	+	+	+	+	++
Ninhydrin test	++	++	++	++	++
NOTE: "-" is absent, "+" is present					

Pharmacological studies

Experimental animals Female wistar rats (Nulliparous and non-pregnant) of 8 weeks to 10 weeks old weighing 200 gms–250 gms supplied by National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22°C ± 30°C and RH between 50 to 60, under artificial lighting 12:12 h light and dark cycle in hygienic condition for at least 5 days prior to the study. The rats were fed with standard laboratory pellet diet (Hindustan lever) and water ad libitum. The studies were performed according to OECD Guidelines 420 and the protocol was approved by the Institutional Animal Ethics Committee).

Anti-inflammatory activity: Anti-inflammatory activity of ethanolic extract of whole plant at doses 100 mg/kg, 200 mg/kg and 400 mg/kg, p.o was studied by 4 different methods.

Carrageenan-induced rat paw edema: The study was conducted according to the method of Winter et al female albino Wistar rats weighing 100 g–250 g were housed in polypropylene cages in a controlled room temperature 22°C+1°C, relative humidity 60%– 70% and with 12 h light and dark cycle [10,11]. The animals were maintained with pellet diet and water ad libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. Five groups of 6 animals each were used for the experiment. Group I of animals were administered with 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. The group II was treated with Indomethacin 20 mg/kg, p.o. Ethanolic extact whole plant of Flemingia chappar 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the, III, IV and V groups of animals respectively. One hour after oral administration, edema was induced by subplantar injection (left hind paw) of 0.1 ml of 1% freshly prepared suspension of carragenan (Sigma Chemical Co., USA) in normal saline to all the animals. The volume of the injected and the contra lateral paws were measured for 3 h his one hour interval after induction of inflammation using Plethysmometer. The percent inhibition of inflammation were calculated by using formula

Where A and B denote mean increase in paw volume of control and drug treated animals respectively.

Histamine induced rat paw edema: In this model edema was induced by sub plantar injection (hind paw) of 0.05 ml of 1% w/v, freshly prepared solution of histamine to

all animals, which were grouped and treated similarly as followed in carragenan induced rat paw edema method. The volume of the injected and the contra lateral paws were measured 3 h after induction of inflammation using Plethysmometer according to the method described by Winter, et al. (1962). In this study Indomethacin (dose 20 mg/kg) was used as standard.

Dextran induced rat paw edema: In this model edema was induced by subplantar injection of 0.05 ml of freshly prepared 1% w/v solution of dextran into the right hind paw of the rats, which were grouped and treated similarly as followed in carragenan induced rat paw edema method [12]. In this study Indomethacin (dose 20 mg/kg) was used as standard.

Cotton wool induced granuloma test: Four groups of 5 animals each were used for the experiment. The rats were anaesthetized under ether anesthesia and 10 mg of sterile cotton pellets were inserted into the axilla of each rat. Group I animals was given 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. The group II was given with the standard drug Indomethacin (20 mg/kg, p.o). Ethanolic extract of whole plant of Flemingia chappar (FCE) at 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the III, IV and V groups of animals respectively. The treatment was continued for 7 consecutive days from the day of cotton pellets implantation. The animals were anaesthetized again on 8th day and the cotton pellets were surgically removed, freed from extraneous tissue; incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the cotton pellets was taken as a measure of granuloma formation [13].

Statistical analysis

All results were expressed as the mean ± SEM. The results were analyzed for statistical significance by one way ANOVA test using computerized GraphPad InStat version 3.05, Graph pad software Inc., San Diego, U.S.A.

Results and Discussion

The phyto chemical analysis of Flemingia chapper was performed in various organic solvents and found to contain carbohydrates, flavonoids, saponins, steroids, peptides and free aminiacids. These molecules in general can serve as potential antioxidants and scavenges of free radicals.

The FCE was performed for its acute toxicity studies, and the concentrations of the 100 mg/kg, 200 mg/kg and 400 mg/kg p.o of whole plant extracts were selected in the study. The results of anti-inflammatory studies using 4 different models were summarized in Table 2. Most of the investigators reported that inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents [13].

Table 2: Anti-inflammatory activity on Carrageenan induced rat paw edema

Group	Treatment	Edema volume (ml)			
		0 h	1 h	2 h	3 h
I	Control	48.67 ± 1.51	54.27 ± 2.41	53.12 ± 2.57	53.7 ± 3.91
II	Indomethacin 20 mg/kg (NSAID)	48.62 ± 2.66	35.23 ± 2.69 (-27.5%)	26.52 ± 1.23 (-45.45%)	22.81 ± 2.03 (-53.08%)
III	FCE 100 mg/kg	48.92 ± 1.16	40.31 ± 1.56 (-17.6%)	36.42 ± 2.65 (-25.55%)	34.52 ± 2.53 (29.43%)
IV	FCE 200 mg/kg	47.56 ± 3.05	37.26 ± 2.63 (-21.65%)	34.42 ± 3.86 (-27.62%)	29.23 ± 2.35 (-38.54%)
V	FCE 400 mg/kg	47.65 ± 2.54	34.21 ± 1.23 (-28.2%)	28.24 ± 2.54 (-40.73%)	25.12 ± 1.85 (-47.28%)

The sub planter injection of carrageenan (1% w/v) to rats have developed edema of high intensity and persisted for 1 h after injection to the control groups. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (27.5%, 17.60%, 21.65%and 28.20%, respectively) of paw edema in groups II to V.

The sub planter injection of carrageenan (1% w/v) to rats have developed edema of high intensity and persisted for 2 h after injection to the control groups. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (45.45%, 25.55%, 27.62% and 40.73%, respectively) in the groups II to V.

Similar to above 2, the sub planter injection of carrageenan (1% w/v) developed edema of high intensity and persisted for 3 h after injection in the control groups. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (53.08%, 29.43%, 38.54% and 47.28%, respectively) in the groups II to V.

The commercial anti-inflammatory drug, Indomethacin has showed maximum of 53.08% of inhibition at the dose of 20 mg/kg p.o. From above table, it was observed that all three concentrations of FCE has inhibited the paw edema of rat from 17.60% to 21.65% and to 28.20% on 1st hour, 25.55% to 27.62% and 40.73% on 2nd hour, and 29.43% to 38.54% and 47.28% on 3rd hour of analysis. Hence the indomethacin 20 mg treatment rat for 3 hours of analysis was almost identical to the data of 400 mg/kg of FCE treatment for 3 hours. Therefore the plant products are showing effective molecules in inhibition of paw edema.

The development of carrageenan induced oedema is bi-phasic. The 1st phase is attributed to the release of histamine, serotonin and kinins whereas, the 2nd phase is related to the release of Leukotrienes [14,15].

The inhibitory action of the drug (FCE) on carrageenan induced paw edema in rats may be mediated through either

any of the mediators alone or in combination. Hence FCE was further investigated against paw edema induced by individual agents like the sub planter injection of Histamine induced rat paw edema showed a maximum inhibition (1% w/v) developed edema of high intensity and persisted for 1 h after injection in the control groups Table 3. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (1.25%, 24.83%,7.11%, 14.80% and 23.03% respectively) in groups II and V.

Table 3: Anti-inflammatory activity on Histamine induced rat paw edema

Group	Treatment	Edema volume (ml)			
		0 h	1 h	2 h	3 h
I	Control	49.26 ± 0.56	47.554 ± 1.25	47.63 ± 2.69	47.30 ± 2.56
II	Indomethacin 20 mg/kg	50.54 ± 1.69	37.99 ± 2.45 (24.83%)	28.21 ± 1.02 (44.18%)	24.74 ± 1.85 (51.04%)
III	FCE 100 mg/kg	45.52 ± 2.56	42.28 ± 1.35 (7.11%	35.27 ± 1.53 (22.51%)	29.72 ± 1.58 (34.71%)
IV	FCE 200 mg/kg	45.24 ± 0.67	38.54 ± 1.52 (14.80%)	32.54 ± 0.89 (28.07%)	25.32 ± 1.43 (44.03%)
V	FCE 400 mg/kg	47.49 ± 1.96	36.55 ± 2.85 (23.03%)	31.69 ± 1.75 (33.27%)	22.53 ± 1.12 (52.55%)

The sub planter injection of Histamine induced rat paw edema showed a maximum inhibition (1% w/v) developed edema of high intensity and persisted for 2 h after injection in the control groups. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (44.18%, 22.51%, 28.07% and 33.27% respectively) in groups II to V.

The sub planter injection of Anti-inflammatory activity of dextran induced albino mice paw edema showed a maximum inhibition (1% w/v) developed edema of high intensity and persisted for 1 h after injection in the control groups table III. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (23.80%, 5.25%, 10.87% and 21.09%, respectively) in group II to V as similar to group II.

After finding the identical effects of FCE, the anti-inflammatory activity of FCE for histamine induced rat paw edema was almost identical to the results. So the inhibition principles of were similar to the effect of antihistamines which reduce the inflammation of rat paw in groups III, IV and V as that of group II.

The sub planter injection of Anti-inflammatory activity of dextran induced rat paw edema showed a maximum inhibition (1% w/v) developed edema of high intensity and persisted for 2 h after injection in the control groups. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (39.57%, 20.21%, 23.40% and 34.34%, respectively) in group III to V similar to group II (Table 4).

Table 4: Anti-inflammatory activity on Dextran induced rat paw edema

Group	Treatment	Edema volume (ml)			
		0 h	1 h	2 h	3 h
I	Control	49.26 ± 1.36	48.29 ± 1.68	46.23 ± 2.26	41.70 ± 2.34
II	Indomethacin 20 mg/kg	50.54 ± 0.36	38.51 ± 2.31 (23.80%)	30.54 ± 2.31 (39.57%)	26.56 ± 1.54 (47.44%)
III	FCE 100 mg/kg	45.52 ± 1.32	43.13 ± 1.25 (5.25%)	36.32 ± 1.54 (20.21%)	30.25 ± 1.32 (33.54%)
IV	FCE 200 mg/kg	45.24 ± 2.30	40.32 ± 1.23 (10.87%)	34.65 ± 1.22 (23.40%)	26.53 ± 1.25 (41.35%)
V	FCE 400 mg/kg	47.49 ± 1.35	37.47 ± 1.65 (21.09%)	31.18 ± 2.66 (34.34%)	25.45 ± 1.76 (46.40%)

The anti-inflammatory activity of FCE for histamine induced rat paw edema was almost identical to the results. So the inhibition principles of were similar to the effect of antihistamines which reduce the inflammation of rat paw in groups III, IV and V as that of group II.

The sub planter injection of Anti-inflammatory activity of dextran induced rat paw edema showed a maximum inhibition (1% w/v) developed edema of high intensity and persisted for 3h after injection in the control groups. The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (47.44%, 33.54%, 41.35%and 46.40%, respectively) in group II to V similar to group II.

All these effects of Table 4 were of identical to Tables 2 and 3. In immunology the cells play main role in defense and when they lose its physiological response due to microbial attack the cells are de-granulated for their self defense. Because of it the de-granulated system induces inflammation. The above 3 studies have elicited anti-inflammatory activity and further to continue the above.

The cotton pellet induced granuloma test was performed using FCE. The results of de-franulation is given in Table 5.The oral administration of FCE at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. showed significant and dose dependent inhibition (61.11%, 36.93%, 52.89% and 59.29%, respectively) in group II to V.

This linearity was continued for 8 days and then levelled off. Therefore, 7 days was chosen for the experiments [16]. Results suggest that the FCE at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. significantly reduced the edema produced by several inducers and are comparable with many standard drugs suggested in each model.

Table 5: Anti-inflammatory activity on Cottonwool induced granuloma

Group	Treatment	Weight of granuloma formation (mg)	% Inhibition
I	Control	80.14 ± 5.78	-
II	Indomethacin 20 mg/kg	31.16 ± 4.45	61.11
III	FCE 100 mg/kg	50.54 ± 4.46	36.93
IV	FCE 200 mg/kg	37.75 ± 5.65	52.89
V	FCE 400 mg/kg	32.62 ± 3.24	59.29

It has been reported by many researchers that flavonoids inhibit arachidonic acid release and eicosanoids synthesis by inhibiting both cyclooxygenase and lipoxygenase activities as well as hamper the non-enzymatic peroxidation of polyunsaturated fatty acids required for the activation of these oxygenase's Quercetin and other flavonoids inhibit leukotrienes synthesis and histamine, prostaglandins release, as well as acts as superoxide scavengers [17-20].

Therefore the molecules present in FCE play an essential role to serve as anti-inflammatory drugs hence it may be a potential constituent to the production of drugs to present lung and nose problems.

Conclusion

Ethanolic extract entire plant of Flemingia chappar (Graham) was systematically evaluated for its anti-inflammatory potential by following standard pharmacological screening methods. Results suggested that the FCE found to possess comparable efficacy with that of standard anti-inflammatory drugs.

Acknowledgement

None.

Conflict of Interest

Authors have no conflict of interest to declare.

References

1. K.M. Anil, Ethnomedicine: A source of complementary therapeutics, Research Signpost, (2010):267–293.
2. B.B. Kakoti, P. Pradhan, S. Borah, K. Mahato, M. Kumar, Analgesic and anti-inflammatory activities of the methanolic stem bark extract of Nyctanthes arbor-tristis linn, Bio Med int, (2013):826295.
3. R. Sobota, M. Szwed, A. Kasza, M. Bugno, T. Kordula, Parthenolide inhibits activation of Signal Transducers and Activators of Transcription (STATs) induced by cytokines of the IL-6 family, Biochem Biophysi Res Commun, 267(2000):329–333.
4. R.N. Mitchell, R.S. Cotran, Robinsons basic pathology, 7th ed, 2000.
5. J.A. DiMasi, R.W. Hansen, H.G. Grabowski, The price of innovation: new estimates of drug development costs, J Health Econ, 22(2003):151–185.
6. A.M. Vittalrao, T. Shanbhag, M. Kumari, K.L. Bairy, S. Shenoy, Evaluation of antiinflammatory and analgesic activities of alcoholic extract of Kaempferia galanga in rats, Indian J Physiol Pharmacol, 55(2011):13–24.
7. CK. Kokate, Practical Pharmacognosy, 2nd ed, 1988.
8. OECD guidelines for the testing of chemicals, section 4/test No. 420: Acute oral toxicity-fixed dose procedure, 2002.
9. OECD guidelines for the testing of chemicals, section 4/test no. 425: Acute oral toxicity: Up-and-down procedure, 2022.
10. OECD guideline for the testing of chemicals adopted by the council: Repeated dose 28-day oral toxicity study in rodents, 1995.
11. N.B. Eddy, D. Leimbach, Synthetic analgesics. II. Dithienyl-butenyl dithienbutylamine, J Pharmacol Exp Ther, 107(1953):385-393.
12. N.K. Jain, S.K. Kulkarni, A. Singh, Role of cystenyl leukotrienes in nociceptive and inflammatory conditions in experimental animals, Eur J Pharmacol, 423(2001):85-92.
13. L.C. Di Stasi, M. Costa, S.L.J. Mendaçolli, M. Kirizawa, C. Gomes, et al. Screening in mice of some medicinal plants used for analgesic purposes in the state of Sao Paulo, J Ethnopharmacol, 24(1988):205.
14. C. Bianchi, J. Franceschini, Experimental observations of Haffner's method for testing analgesic drugs, Br J Pharmacol, 9(1954):280-284.
15. R. Koster, W. Anderson, E. Beer, Acetic acid for analgesic screening, Fed Proc, 18(1959):412.
16. C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin induced oedema in hind paw of rats as an assay for anti–inflammatory drugs, Proc Soc Exp Bio Med, 111(1962):544-7.
17. C.C. Lin, W.C. Lin, C.H. Chang, T. Namba, Anti–inflammatory and hepatoprotective effects of Ventilago leiocarpa. Phtoth Res, 9(1994):11–15.
18. S.C. Mandal, A. Kumar, S. Mohana Lakshmi, S. Sinha, T. Murugesan, et al. Anti-tussive activity of Asparagus racemosus root against sulphur dioxide induced cough in mice, Fitoterapia, 71(2000):687-689.
19. P.K. Smith, W.E. Hambourger, The ratio of the toxicity of acetanilide to its antipyretic activity in rats, J Pharmacol Exptl Ther, 54(1935):346-349.

20. B.J. Divya, K. Thyagaraju, Bioactivity of Mucuna prurienus and Allium sativum on cyclooxygenases biochemical characterization of herbal formulation, 2020.