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# Anti-inflammatory Activity of *Abelmoschus manihot* Extracts

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**Abstract:** The present study was carried out to evaluate the anti-inflammatory activity of the petroleum ether and methanol extract of *Abelmoschus manihot* (Malvaceae) woody stems using paw edema model. The air-dried, powdered woody stems (900 g) were extracted over Soxhlet with petroleum ether and methanol. The crude dried petroleum ether (12 g) and methanol (18 g) extracts was prepared at the doses of 100, 200 and 400 mg $kg^{-1}$ and evaluated for anti-inflammatory using the carrageenan and histamine-induced paw edema test. The results obtained indicate that the extracts possessed significant ($p<0.01$) anti-inflammatory activity, which was found to be dose-dependent. This study showed that the petroleum ether and methanol extracts of *Abelmoschus manihot* woody stems possess potential pharmacological active constituents responsible for inhibition of the inflammation effect.

**Key words:** *Abelmoschus manihot*, anti-inflammatory, paw edema, rats, drug

## INTRODUCTION

Inflammation is defined as local response of living mammalian tissue, to injury due to any agent or Inflammation is reaction of vascular and supporting element of a tissue to injury and result in formation of protein rich exudates, provided the injury has not been so severe as to destroy the area. It is a common clinical condition said to be protective response intended to eliminate the initial cause of cell injury as well as necrotic cells and tissues resulting from original insult. Inflammation manifests usually in form of painful swelling associated with some changes in skin covering the site and usually process may interfere with physical function of inflamed part. The cardinal signs viz., redness, swelling, heat, pain and loss of function are brought about by complex actions of various inflammogens e.g., histamine, bradykinins, prostaglandins (PGs), etc. The inflammatory response could be suppressed by logically, by inhibiting the activity of these endogenous mediators. However, use of antagonists of some of mediators to suppress inflammation may not be feasible clinically. Antihistamines are rarely used as anti-inflammatory agents probably due to their clinical inefficacy, while PG synthesis inhibitors like NSAIDs are clinically effective and are widely used in practice.

*Abelmoschus manihot* Linn. (Malvaceae) is a large annual erect hairy plant, 1.2-1.8 m high. It is native to China, was introduced into India, near Calcutta and in coastal areas of Maharashtra. The plant yields a tough, light cream, non-lustrous fibre (10%) which resembles jute in color. The seeds yield fatty oil (10.8%). The oil contains 19% of saturated acids and 81% of liquid acids. The mucilage contains polysaccharides and proteins (Kiritikar and Basu, 1994). The flower contains quercetin-3-robinoside, quercetin-3'-glucoside, hyperin, myrecetin and anthocyanins (Lai *et al*., 2006). The different chromatographic methods have been developed on the flavones present in the plant (Liang *et al*., 2007; Lai *et al*., 2009; Yi *et al*., 2008). The flowers are used in the treatment of chronic bronchitis and toothache. The ethanol extract of flower was screened for antiviral activity and it was observed that the hyperoside shown significant anti HBV activity (Lin-Lin *et al*., 2007). The flavones present in the plant showed preventive effect in the injury (Liu *et al*., 2009; Wen and Chang, 2007). The leaves were tested on bone loss in ovarectomised rats and it was observed that it was able to prevent the ovariectomy-induced femoral osteopenia (Puel *et al*., 2005). The modulatory effect of total flavone of *Abelmoschus manihot* (TFA) on NMDA-activated current ($I_{NMDA}$) was investigated in cultured rat hippocampal neurons using the whole-cell patch-clamp technique. TFA rapidly and reversibly inhibited the $I_{NMDA}$ in a concentration-dependent manner (Xin-Ping *et al*., 2006). This study, evaluated the anti-inflammatory activity of the woody stems of *Abelmoschus manihot*.

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## MATERIALS AND METHODS

**Plant material:** The bark of *Abelmoschus manihot* was collected in August 2007 from Toranmal Hills of Maharashtra and authentication of the plant specimen was established by Patil (2003) was deposited in our departmental herbarium for future reference.

**Preparation of extracts:** The woody stems were chopped into small pieces, air-dried and powdered. The powdered plant material was successively soxhlet extracted with petroleum ether and methanol at room temperature for 48 h. The percentage of yield of the petroleum ether, chloroform and methanol extracts was 0.65, 0.73 and 2.50%, respectively.

**Standard drugs/chemicals solutions:** Diclofenac sodium (Lupin) (10 mg $kg^{-1}$), used for the purpose of comparison, was prepared by dissolving, in saline: CMC. Carrageenan (λ4) (C3889-5G), Histamine (H-7375), were obtained from Sigma Aldrich, USA.

**Pharmacological studies**

**Animals:** Wistar rats (150-200 g; 8-11 weeks old) and Swiss albino mice (25-30 g; 7-10 weeks old) were, obtained from R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur and Government Veternary College, Mahu, MP (India). The animals were housed in Animal house of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India in polycarbonate cages, in a room maintained, under controlled room temperature 22±2°C, relative humidity 60-70% and provided with food and water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout. All studies were carried out by using 6 animals in one group for anti-inflammatory activity.

**Acute toxicity test:** Acute toxicity tests were performed according to OECD (2006) guidelines. Animals were weighed and marked, a single high dose, 2000 mg $kg^{-1}$ of petroleum ether extract and methanol extract as recommended by the OECD guidelines was administered to the first animal. After a single administration, a sign of toxicity and behavior was observed each hour up to the 24 h. If this animal was dying, then one lesser dose of that dose was administered to the next animal. Same procedure was followed for that animal. If the animal survives, then the same dose was given to the next 5 animals. All the animals were observed for the signs of toxicity and mortality for up to the 14 days.

Additional observations like changes in skin, eyes and mucous membranes and also respiratory circulatory, autonomic and central nervous system and behavior pattern. Attention was also given to observed precipitation of tremors and convulsions.

**Carrageenan-induced paw edema:** Wistar rats of either sex (150-200 g) were divided into eight groups containing 6 animals in each. The rats were fasted for 24 h prior to induction of edema however water was available *ad libitum*. Rats were deprived of water only during the experiment to ensure uniform hydration and minimize variability in edematous response. Inflammation of hind paw was induced by injecting 0.1 mL of 1% w/v carrageenan in normal saline into the subplantar region of right hind paw (Winter *et al*., 1962). The negative control group received Saline: CMC (0.5%) solution (Moraes *et al*., 2007) and the positive control group received Diclofenac sodium (10 mg $kg^{-1}$) p.o., (Saneja *et al*., 2009). Three groups orally received petroleum ether extract at the doses 100, 200 and 400 mg $kg^{-1}$, respectively. The remaining three groups orally received methanol extract at doses 100, 200 and 400 mg $kg^{-1}$, respectively. All the drug treatments were given 1 h before the carrageenan injection; edema was expressed as the increase in paw volume due to carrageenan injection. The paw volume was measured with a digital plethysmometer (Ugo Basile, 7140) before and 1, 2, 3, 4, 5 and 6 h after carrageenan injection (Amresha *et al*., 2007; Gupta *et al*., 2005). The extracts and the reference drug were dissolved in 0.5% carboxy methyl cellulose solution just before use.

**Histamine induced paw:** Wistar rats of either sex weighing 150-200 g were divided into eight groups containing 6 animals each. The rats were fasted for 24 h prior to induction of edema however water was available *ad libitum*. Inflammation of hind paw was induced by injecting 0.1 mL of histamine (1 mg $mL^{-1}$) in normal saline into the subplantar region of right hind paw (Gupta *et al*., 2005). The negative control group received CMC (0.5%) solution (Moraes *et al*., 2007) and the positive control group Diclofenac sodium (10 mg $kg^{-1}$) p.o., (Saneja *et al*., 2009). The last section of the methods for histamine detailly. Percentage rise in paw volume was determined by the formula stated below.

$$\text{Rise (\%)} = \frac{vt - vc}{vc} \times 100$$

Where:
Vt = Paw volume post carrageenan injection t
Vc = Paw volume before carrageenan injection o

**Data statistical analysis:** The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using graph pad in stat 3 Demo and obtained in the study were compared with the vehicle control group. The p-values<0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

**Acute toxicity:** *Abelmoschus manihot* woody stem extracts did not produce any mortality even at the dose of 2000 mg $kg^{-1}$, p.o., *A. manihot* was thus found to be non-toxic, the results showed no clinical signs and mortality of the animal therefore an $LD_{50}$ >2000 mg $kg^{-1}$ b.wt. may be assumed. On the basis of above results, three doses (100, 200, 400 mg $kg^{-1}$, p.o.) of *A. manihot* was selected for further pharmacological studies.

**Carrageenan-induced rat paw edema:** The petroleum ether and methanol extracts at the doses of 200 and 400 mg $kg^{-1}$ p.o., showed very good results and caused a significant inhibition in the percent rise of carrageenan induced rat paw edema (Table 1). The maximal inhibition in the percent rise of edema volume was achieved at the dose of 400 mg $kg^{-1}$ ($p<0.01$) of petroleum ether and methanol extracts, when compared to standard drug diclofenac sodium (10 mg $kg^{-1}$).

**Histamine induced rat paw edema:** The petroleum ether and methanol extracts at the doses of 200 and 400 mg $kg^{-1}$ p.o., showed very good results and caused significant inhibition in the percent rise of Histamine induced rat paw edema (Table 2). The maximal inhibition in the percent rise of edema volume was achieved at the dose of 400 mg $kg^{-1}$ ($p<0.01$) of petroleum ether and methanol extracts, when compared to standard drug diclofenac sodium (10 mg $kg^{-1}$).

The present study indicates that *Abelmoschus manihot* has the pharmacological potential as an anti-inflammatory agent when tested on various animal models. Although, the present study did not aim at isolation and identification of bioactive compounds, the phytochemical screening of petroleum ether and methanol extracts demonstrated the presence of flavonoids, steroids, triterpenoids, which are suggested to act synergistically to exert the observed pharmacological activity (Maj and Rogóż, 2000), the presence of steroids and flavonoids in petroleum ether and methanol extract could possibly lead to the observed activities. The anti-inflammatory activity of petroleum ether and methanol extracts could also be linked to the ability of the extract to inhibit prostaglandin synthesis (Chan *et al.*, 1995). This fact is supported by claims that the carrageenan-induced inflammation is a COX-dependent response and is more effectively controlled with arachidonate cyclo-oxygenase (Ballou *et al.*, 2000) but not arachidonate lipo-oxygenase inhibitors (Gamache *et al.*, 1986).

To demonstrate whether petroleum ether and methanol extracts is producing anti-inflammatory activity

Table 1: The anti-inflammatory activity of petroleum ether and methanol extracts using the carrageenan-induced paw edema test

| | | Percentage increase in paw edema (h) | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | Dose (mg $kg^{-1}$) | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | | 51±3.97 | 69.0±4.44 | 91.4±1.98 | 81.9±4.75 | 83.1±3.78 | 84.5±4.22 |
| Petroleum ether extract | 100 | 36.1±1.92 | 54.5±2.00 | 64.0±3.78 | 53.4±2.96** | 50.7±2.70 | 41.0±4.40** |
| | 200 | 31.1±5.49** | 40.4±1.46* | 57.9±4.22** | 44.2±2.49** | 48.9±3.15** | 38.0±6.3** |
| | 400 | 30.1±4.17** | 42.8±5.89** | 69.6±6.26** | 41.5±6.75** | 43.5±5.47** | 25.8±7.05** |
| Methanol extract | 100 | 36.5±6.73* | 49.2±10.4 | 62.1±8.06 | 58.5±6.23 | 73.8±5.03 | 67.5±5.24 |
| | 200 | 39.3±3.79* | 46.0±2.74 | 82.4±4.95 | 46.0±4.2* | 59.3±4.87** | 57.4±1.42** |
| | 400 | 25.5±2.13** | 39.6±4.19** | 84.7±7.54* | 47.0±6.9 | 65.7±5.55 | 60.9±6.76* |
| Diclofenac | 10 | 16±1.01** | 18.1±0.49** | 44.3±1.82** | 36.0±3.6** | 33.2±4.83** | 28.6±4.05** |

Values represent Mean±SEM, n = 6. One way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01 compare with control group

Table 2: The anti-inflammatory activity of Petroleum ether and methanol extracts assessed using the histamine-induced paw edema test

| | | Percentage increase in paw edema (h) | | |
|---|---|---|---|---|
| Treatments | Dose (mg $kg^{-1}$) | 1 | 2 | 3 |
| Control | | 56.2±3.02 | 72.6±2.62 | 76.6±4.32 |
| Petroleum ether extract | 100 | 41.2±2.14* | 40.2±2.76** | 37.5±2.90** |
| | 200 | 28.0±2.46** | 26.5±0.893** | 22.9±1.44** |
| | 400 | 21.8±5.09** | 15.9±2.40** | 13.3±2.21** |
| Methanol extract | 100 | 37.0±1.55** | 31.4±1.14** | 25.5±0.940** |
| | 200 | 41.1±4.60* | 36.8±4.76** | 29.4±4.78** |
| | 400 | 40.4±5.47** | 30.1±6.37** | 25.1±5.99** |
| Diclofenac | 10 | 12.7±0.835** | 11.64±1.18** | 10.13±0.954** |

Values represent Mean±SEM, n = 6. One way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01 compare with control group

by acting on histamine, the effect of petroleum ether and methanol extracts was studied on histamine induced inflammation.

Histamine induced rat paw inflammation is the model used to study the anti-inflammatory activity of various agents. Histamine is one of the important mediators of inflammation. Histamine increase vascular permeability and act with prostaglandins to induce edema (Singh *et al*., 2003; Vasudevan and Parle, 2006). These mediators are stored in the secretory granules and are released from mast cells during their activation.

In histamine induced rat paw inflammation model petroleum ether and methanol extracts and reference drug Diclofenac sodium significantly $p<0.01$ decreased the inflammation at 1st h after histamine injection. At the second hour petroleum ether and methanol extracts caused decrease in paw edema and also decrease at third hour then decreases slowly (Londonkar *et al*., 2010). The present results support the ethno-medical application of *Abelmoschus manihot* woody stem in the treatment of inflammation diseases. Further experimentation is needed in order to understand the precise mechanism of action in anti-inflammatory activities by the extracts.

## CONCLUSION

The present study demonstrated that the petroleum ether and methanol extracts possessed anti-inflammatory activity. Thus, the present study confirmed the folklore use of *A. manihot* fruit for the treatment of various ailments and the plant's potential pharmacological activities merit further investigation.

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## REFERENCES

Amresha, G., G.D. Reddy, C.H.V. Rao and P.N. Singh, 2007. Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. J. Ethnopharmacol, 110: 526-531.

Ballou, L.R., R.M. Botting, S. Goorha, J. Zhang and J.R. Vane, 2000. Nociception in cyclooxygenase isozyme-deficient mice. Proc. Nat. Acad. Sci. USA., 97: 10272-10276.

Chan, T.F., H.Y. Tsai and W. Tian-Shang, 1995. Anti-inflammatory and analgesic activities from the roots of *Angelica pubescens*. Plant Med., 61: 2-8.

Gamache, D.A., J.T. Povlishock and E.F. Ellis, 1986. Carrageenan-induced brain inflammation. Characterization of the model. J. Neurosurg, 65: 679-685.

Gupta, M., U.K. Mazumder, R.K. Sambath, P. Gomathi, Y. Rajeshwar, B.B. Kakoti and V.S. Tamil, 2005. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. J. Ethnopharmacol., 98: 267-273.

Kiritikar, K.R. and B.D. Basu, 1994. Indan Medcid Plants. 2nd Edn., University of Allahabad, Allahabad India, pp: 1606-1609.

Lai, X.Y., Y.Y. Zaho and H. Laing, 2006. Studies on chemical constituents in flower of *Abelmoschus manihot*. Zhongguo Zhong Yao Za Zhi, 31: 1597-1600.

Lai, X., H. Liang, Y. Zaho and B. Wang, 2009. Simultaneous determination of seven active flavonols in the flowers of *Abelmoschus* manihot by HPLC. J. Chromatogr. Sci., 47: 206-210.

Liang, H., X. Lai, Y. Zaho, Y. Bai, B. Wang and D. Gao, 2007. SPE-HPLC method for the determination of four flavonols in rat plasma and urine after oral administration of *Abelmoschus* manihot extract. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 852: 108-114.

Lin-Lin, W.U., Y. Xin-Bo, H. Zheng-Ming, L. He-Zhi and W.U. Guang-Xia, 2007. *In vivo* and *in vitro* antiviral activity of hyperoside extracted from *Abelmoschus manihot* (L.). Med. Acta Pharmacol. Sin., 28: 404-409.

Liu, M., Q.H. Zaing, J.L. Hao and L.L. Zahou, 2009. Protective effect of total flavones of *Abelmoschus manihot* L. Medic against poststroke depression injury in mice and its action mechanism. Anat Rec., 292: 412-422.

Londonkar, R., A. Kamble and V.C. Reddy, 2010. Antiinflammatory activity of pandanus odoratissimus extract. Int. J. Pharmacol, 6: 311-314.

Maj, J. and Z. Rogóż, 2000. Synergistic effect of amantadine and imipramine in the forced swimming test. Pol. J. Pharmacol, 52: 111-114.

Moraes, B.M., C.D. Amaral, M.S.S. Morimoto, C.L.G. Vieira, F. F. Perazzo and J.C.T. Carvalho, 2007. Anti-inflammatory and analgesic actions of etoricoxib (an NSAID) combined with misoprostol. Inflammopharmacology, 15: 175-178.

OECD, 2006. Guidelines for testing of chemicals. Revised Draft Guidelines 425: Acute Oral Toxicity-Up and Down Method.

Patil, D.A., 2003. Flora of Dhule and Nandurbar districts (Maharashtra). Bishen Singh Mahendra Pal Singh, Dehradun, ISBN: 81-211-0164-6, pp: 696.

Puel, C., J. Mathey, M.J. Davicco, P. Lebecque, B. Chanteranne, M.N. Horcajada and V. Coxam, 2005. Preventive effect of *Abelmoschus manihot* (L.) medik on bone loss in the overiectomised rats. J. Ethnopharmacol., 99: 55-60.

Saneja, A., D. Kaushik, S.L. Khokra, P. Kaushik, C. Sharma and K.R. Aneja, 2009. Evaluation of activities of Mitragyna parvifolia fruit extract. J. Nat. Prod., 2: 49-54.

Singh, B., M.K. Sharma, P.R. Meghwal, P.M. Sahu and S. Singh, 2003. Antiinflammatory activity of shikonin derivatives from *Arnebia hispidissima*. Phytomedicine, 10: 375-380.

Vasudevan, M. and M. Parle, 2006. Pharmacological actions of *Thespesia populnea* relevant to *Alzheimers disease*. Phytomedicine, 13: 677-687.

Wen, J.Y. and Z.W. Chang, 2007. Protective effect of pharmacological preconditioning of total flavones of abelmoschl manihot on cerebral ischemic reperfusion injury in rats. Am. J. Chin Med., 35: 653-661.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547.

Xin-Ping, C., Q. Song, D. Liu-Yi and Z. Jiang-Ning, 2006. Inhibitory effect of total flavone of *Abelmoschus manihot* L. medic on NMDA receptor-mediated current in cultured rat hippocampal neurons. Neurosci. Res., 55: 142-145.

Yi, L., L. Wujie, L.X.L. Xiaomei, L. Yuzhen, Z.Z. Qingying and Z. Yuying, 2008. Simultaneous determination of the active ingredients in *Abelmoschus manihot* (L.) Medicus by CZE. Chromatographia, 67: 819-823.