# Anti-inflammatory activity of aqueous methanolic extract of *Swietenia mahagoni* (L.) Jacq. (Meliaceae) leaves

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## SUMMARY

Pharmacological investigations were carried out with aqueous methanolic extract (AME) of *Swietenia mahagoni* (L.) Jacq. (Meliaceae) leaves. Acute toxicity studies revealed that the $LD_{50}$ dose of AME was 600 mg/kg, i.p. AME was found to possess significant anti-inflammatory activity in acute, sub-chronic and chronic models of inflammation. AME selectively inhibited cyclooxygenase (COX)-2 activity, which is involved in arachidonic acid metabolism and biosynthesis of prostaglandins under inflammatory conditions. Treatment with AME significantly enhanced total peritoneal cell count and the number of macrophages in normal mice, which revealed that AME may also alter the immune response along with its anti-inflammatory effect. The saponins or the alkaloids present in AME may be responsible for the anti-inflammatory activity.

**Key words:** Swietenia mahagoni leaf; Anti-inflammatory activity; Cyclooxygenase-2 inhibition

## INTRODUCTION

Leaves of medicinal plants are common ingredients of many folk and herbal medicines (Subapriya and Nagini, 2005; Guo *et al.*, 2006) and leaf extracts of a number of medicinal plants have been reported to possess pharmacological activity, including anti-inflammatory activity (Ojewole, 2005; Owoyele *et al.*, 2005; Sertie *et al.*, 2005). *Swietenia mahagoni* (L.) Jacq. (Meliaceae) is a humid zone species, with natural distribution in the Caribbean region, and has been extensively planted in South Asia and in the pacific. It is an evergreen to semi-evergreen tree of 30 - 35 m height. The leaves are clustered, glabrous, 12 - 15 cm long, peripinnately compound with 2 - 4 pairs of leaflets (ovate-lanceolate, 5 - 6 cm long, 2 - 3 cm wide, dark green, glabrous). The tree has potential use for large-scale timber production due to its excellent timber quality. It is also used in agro forestry for soil improvement and as an ornamental tree (Schmidt and Joker, 2000). The seeds of this plant have been reported to contain triterpenoids possessing anti-platelet activity and anti-microbial activity (Ekhimoto *et al.*, 1991). Since the plant belongs to the same Meliaceae family as neem (Azadirachta indica), it is expected that it might also be a storehouse of many chemicals of medicinal and pharmacological interest. The purpose

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of the present work was to investigate the mechanism of anti-inflammatory activity of aqueous methanolic extract (AME) of *Swietenia mahagoni* (L.) Jacq. leaves.

## MATERIALS AND METHODS

**Plant material**

The leaves of *Swietenia mahagoni* (L.) Jacq. were collected by local supplier from sites around Kolkata, India, during June, 2006. The plant was identified by Indian Botanical Garden, Howrah, India, where a voucher specimen (SR-001) has been kept.

**Extraction and preparation of test sample**

One kg of leaves of *Swietenia mahagoni* (L.) Jacq. were sun dried, ground and soaked in three liters of 50% aqueous methanol for one week at room temperature (28 - 34 °C) with occasional shaking (Ekpendu *et al.*, 1994; Manga *et al.*, 2004; Gulfraz *et al.*, 2006). The supernatant was filtered and to the residue 1 liter of 50% aqueous methanol was added and kept for another one week. The methanol portion in the whole supernatant was evaporated using a rotary evaporator and then it was lyophilized to remove the water. The solid dark brown residue (30 g) thus obtained was designated as 'AME' and was kept at 4 °C. To make a stock solution, 100 mg of the AME was dissolved in 1 ml distilled water from which different suitable doses were prepared for intraperitoneal administration to different experimental animals.

**Phytochemical screening**

AME was screened qualitatively for various constituents like alkaloids, steroids, flavonoids, saponins, reducing sugars, tannins and anthraquinones using established methods (Okerulu and Ani, 2001).

**Animals**

Experiments were performed on albino (Sprague Dawley strain) rats weighing 100 - 125 g and Balb C mice weighing 20 - 25 g. The animals were bred in the institute's animal house and were housed under conditions of 24 ± 2 °C and 55 - 60% relative humidity and 12 h light and 12 h dark cycle. During maintenance, the animals received a diet of food pellets (fortified with minerals and vitamins) prepared in the animal house and water ad libitum. All animal studies were carried out after getting clearance from Institute animal ethics committee.

**Chemicals**

Arachidonic acid, carrageenan, croton oil, Freund's complete adjuvant, ibuprofen and indomethacin were purchased from Sigma; acetic acid from E. Merck; phenidone from Biomol Co; COX inhibitor screening assay kit and Lipoxygenase inhibitor screening assay kit from Cayman chemical; Biotrak enzyme immunoassay kit RPN222 and Biotrak enzyme immunoassay kit RPN221 from Amersham Biosciences Inc. and all other chemicals were of analytical grade purchased locally.

**Acute toxicity studies**

Different doses of AME (50 mg to 1 g/kg) were administered intraperitoneally to groups of mice (n = 10). The $LD_{50}$ (24 h) was calculated according to Ghosh (1984).

**Anti-inflammatory studies**
**Acute models**
**Carrageenan induced paw oedema in rats**

The animals were divided into five groups (n = 6). The first group served as negative control and received normal saline (0.1 ml/100 g, i.p.), the second group was administered the standard drug, ibuprofen (100 mg/kg, i.p.), the other three groups received AME at doses of 30, 60 and 120 mg/kg, i.p. respectively. Thirty minutes later oedema was induced by injecting carrageenan (0.1 ml/100 g from a 10 mg/ml solution) into the plantar aponeurosis of right hind paw of each rat (Winter *et al.*, 1962). The left hind paw served as the control. After 4 h, the rats were sacrificed by cervical dislocation and the paw volume was

measured using a plethysmometer (UGO BASILE) and in each case the volume of the right hind paw was compared with that of the corresponding left hind paw.

**Croton oil induced ear inflammation in mice**
Croton oil irritant solution prepared according to Brooks *et al.* (1985) was applied (0.1 ml) to the inner surface of the right ear of each animal. The mice were divided into five groups (n = 6). The first group served as negative control and received 0.1 ml/10 g of normal saline, the animals of the second group received ibuprofen (100 mg/kg, i.p.) and mice of groups three, four and five were given 30, 60 and 120 mg/kg, i.p. of AME respectively 30 min before the application of croton oil. After 4 h the mice were sacrificed by cervical dislocation and 7 mm punches were made in the ears of all the animals using a cork borer. The weight of each ear disc was measured and comparisons were done with that of the control group.

**Sub-chronic model**
**Cotton pellet induced granuloma formation in rats**
Two autoclaved cotton pellets, each weighing 10 ± 1 mg, were implanted subcutaneously into both sides of the groin region of each rat (D'Arcy *et al.*, 1960). The animals were divided into five groups (n = 6). The first group served as negative control and received normal saline daily at a dose of 0.1 ml/100 g body weight i.p. for 7 days, the second group received ibuprofen daily at a dose of 100 mg/kg, i.p for 7 days and AME at doses of 30, 60, 120 mg/kg, i.p were injected daily into the rats of groups 3, 4 and 5 respectively for 7 days. After 7 days the rats were sacrificed by cervical dislocation, the cotton pellets along with the granuloma tissues were carefully taken out, dried in an oven at 60 °C, weighed and compared with control (D'Arcy *et al.*, 1960).

**Chronic model**
**Freund's adjuvant induced polyarthritis in rats**

The rats were divided into five groups (n = 6). 0.1 ml of Freund's complete adjuvant was injected into the plantar pads of each animal on day 1. While the first group served as the negative control and received 0.1 ml/100 g, i.p. normal saline, those of the second group received the standard drug ibuprofen at a dose of 100 mg/kg, i.p. and the rats of groups 3, 4 and 5 were injected with AME at doses of 30, 60 and 120 mg/kg, i.p. respectively for 21 consecutive days. The paw volume of each rat was measured on day 0 before adjuvant administration and on day 21 after treatment using plethysmometer (UGO BASILE). Inhibition in inflammation was thereby calculated. Severity and development of secondary lesions were also compared (Newbould, 1963).

**Possible mechanism of action**
**Arachidonic acid induced paw oedema in rats**
The animals were divided into six groups (n = 6). Group I served as negative control and received 0.1 ml/100 g, i.p. of normal saline, group II received the standard cyclooxygenase blocker, indomethacin (10 mg/kg, p.o.), group III received the standard dual blocker, phenidone (200 mg/kg, i.p.) and the other three groups were given AME at doses of 30, 60 and 120 mg/kg, i.p. respectively. Paw oedema was induced by single injection of 0.1 ml of 0.5% arachidonic acid in 0.2 M carbonate buffer (pH 8.4) into the right hind paw (sub plantar) of each rat 1 h after drug and AME administration. Hind paw volume was measured in each case one h after arachidonic acid injection (DiMartino *et al.*, 1987).

**Determination of COX-1 and COX-2 enzyme inhibitory activity in cell free system**
The COX-1 (ovine) and COX-2 (human recombinant) inhibitory assay was carried out using a COX Inhibitor Screening Assay Kit (Cayman Chemicals) according to the manufacturer's protocol. Briefly, COX enzymes, COX-1 and COX-2, respectively and heme were added to test tubes containing COX reaction buffer (0.1 M Tris–HCl, pH 8.0, containing 5 mM EDTA

and 2 mM phenol). The mixture was vortexed and exposed to either reaction buffer or different concentrations of AME and incubated for 10 min at 37 °C. Subsequently, to initiate the reaction, arachidonic acid solution was added, vortexed and incubated for 2 min at 37 °C. HCl (1 M) was then added to stop the enzyme catalysis followed by chemical reduction of prostaglandin (PG) PGH2 to PGF2α with saturated stannous chloride solution for 5 min at room temperature. The COX activity was measured based on the amount of PGF2α generated in the reaction tube and detected by the enzyme immunoassay (EIA) kit using a standard curve. The COX-1 and COX-2 enzyme inhibitory activities of AME were thereby determined.

**Determination of LOX enzyme inhibitory activity in cell free system**

The 15-lipoxygenase (soybean) inhibition assay was carried out using a Lipoxygenase Inhibitor Screening Assay Kit (Cayman Chemicals), according to the manufacturer's protocol. Briefly, 15-LO was exposed to assay buffer (0.1 M Tris-HCl, pH 7.4) or different concentrations of AME. Arachidonic acid was added to start the reaction and kept on a shaker for 5 min. Chromogen was added to stop enzyme catalysis and develop the reaction. After 5 min, the absorbance was read at 490 nm and the LOX enzyme inhibitory activity of AME was thereby calculated.

**Human whole blood assay**

**COX-1 assay [determination of thromboxane B2 (TxB2) production]**

Blood (5 ml) was collected from a healthy male volunteer of 28 years into vacutainers containing no anticoagulants. Whole blood was dispensed into glass tubes containing AME or DMSO. The tubes were transferred to an incubator, and the blood was allowed to clot (37 °C, 95% air, 5% $CO_2$). After 30 min., blood clots were released from the walls of the tubes and incubated for a further 30 min. Serum was separated by centrifugation at

3000 rpm for 10 min. Levels of Thromboxane B2 (TxB2) were determined by EIA (Biotrak enzyme immunoassay kit RPN221 from Amersham Biosciences Inc.) according to manufacturer's protocol.

**COX-2 assay (determination of PGE2 Production)**

Heparinized blood (collected from the same donor) was immediately divided into aliquots and was incubated with aspirin (100 μM) for 6 h at 37 °C to allow aspirin to inactivate COX-1 irreversibly. Further incubation in the presence or absence of LPS (10 μg/ml), DMSO or AME was done for 18 h at 37 °C. Plasma was separated by centrifugation at 3000 rpm for 10 min. Plasma PGE2 levels were determined by EIA (Biotrak enzyme immunoassay kit RPN222 from Amersham Biosciences Inc.) according to manufacturer's protocol.

**Effect of extract on normal peritoneal cells**

Ninety six mice were divided into four groups of 24 mice in each group. While group I received 0.1 ml/10 g, i.p. normal saline, groups II, III and IV received 30, 60 and 120 mg/kg, i.p. of AME respectively. Total peritoneal exudate cells were counted from each group (6 mice at a time) at 6, 12, 24 and 48 h of treatment and compared (Hudson and Hay, 1989). The number of phagocytes was also determined by staining with 1% neutral red solution using a haemocytometer (Hudson and Hay, 1989).

**Statistical analysis**

Data are reported as arithmetic mean ± S.E.M. Statistical analysis was done by one way analysis of variance (ANOVA) followed by Dennett's test or by Student's *t*-test. A probability value of less than 0.05 was chosen as the criterion of statistical significance.

## RESULTS

**Phytochemical screening**

AME showed positive reactions for alkaloids, saponins and reducing sugars but tested negative for steroids, flavonoids, tannins and anthraquinones.

### Acute toxicity studies

The $LD_{50}$ of AME was found to be 600 mg/kg, i.p.

### Anti-inflammatory studies
### Acute models
### Carrageenan induced paw oedema in rats

AME significantly inhibited carrageenan induced paw oedema as compared to control. A dose-dependent inhibition in inflammation was observed which was comparable with the standard antiinflammatory drug, ibuprofen. The percent inhibition produced by AME at doses of 30, 60 and 120 mg/kg, i.p. were 76%, 85% and 89% respectively (Table 1).

### Croton oil induced ear inflammation in mice

AME at doses of 60 and 120 mg/kg, i.p. significantly inhibited ear inflammation comparable with ibuprofen. Reduction in inflammation caused by the lowest dose i.e., 30 mg/kg, i.p. of AME used in the study was found to be statistically insignificant (Table 1).

### Sub chronic model

Cotton pellet induced granuloma formation in rats
AME significantly inhibited the granuloma formation

in a dose-dependent manner. When compared to control, the percent inhibition of the increase in dry weight of cotton pellet granuloma were 52%, 59% and 60% at doses of 30, 60 and 120 mg/kg, i.p. of AME respectively and this inhibition was more than the 36% inhibition produced by ibuprofen (Table 1).

### Chronic model
### Freund's adjuvant induced polyarthritis in rats

Treatment with AME at doses of 30, 60 and 120 mg/kg, i.p. significantly inhibited the development of arthritis by 63%, 81% and 83% respectively in a dose-dependent way whereas ibuprofen reduced the development of arthritis by 66% as compared to control (Table 1). When comparisons were made with that of control, it was found that the formation of secondary lesions like tail and ear nodes was also inhibited after treatment with AME for consecutive 21 days.

### Possible mechanism of action
### Arachidonic acid induced paw oedema in rats

Treatment with the dual blocker, phenidone (200 mg/kg, i.p.) significantly inhibited arachidonic

**Table 1.** Effect of AME on four different models of inflammation

| Treatment group | Dose (mg/kg i.p) | Carrageenan induced paw oedema (ml) | Croton oil induced ear inflammation (mg) | Increase in cotton pellet wt. (mg) | Freund's adjuvant induced arthritis (ml) |
|---|---|---|---|---|---|
| N.Saline | Control | 4.75 ± 0.33 | 10.6 ± 2.53 | 49.44 ± 3.63 | 3.42 ± 0.54 |
| Ibuprofen | 100 | 1.14 ± 0.21* | 8 ± 0.40* | 31.63 ± 1.66* | 1.67 ± 0.28* |
| AME | 30 | 1.14 ± 0.09* | 10.2 ± 0.18 | 23.6 ± 1.50* | 1.25 ± 0.28* |
| AME | 60 | 0.71 ± 0.10* | 8.5 ± 0.61* | 20.43 ± 1.76* | 0.67 ± 0.21* |
| AME | 120 | 0.5 ± 0.11* | 6.7 ± 0.59* | 19.8 ± 2.90* | 0.58 ± 0.08* |

Data are mean ± S.E.M. *denotes significant inhibition as compared to control ($P < 0.05$).

**Table 2.** Effect of AME on arachidonic acid induced paw oedema in rats

| Treatment group | Dose | Arachidonic acid induced paw oedema (difference in paw volume in ml) |
|---|---|---|
| N.Saline | 0.1 ml/100 g, i.p. | 3.75 ± 0.25 |
| Indomethacin | 10 mg/kg, p.o. | 2 ± 0.45* |
| Phenidone | 200 mg/kg, i.p. | 0.75 ± 0.14* |
| AME | 30 mg/kg, i.p. | 1.75 ± 0.32* |
| AME | 60 mg/kg, i.p. | 1.5 ± 0.4* |
| AME | 120 mg/kg, i.p. | 0.875 ± 0.239* |

Data are mean ± S.E.M. *denotes significant inhibition as compared to control ($P < 0.05$).

acid induced paw oedema in rats by 80% whereas indomethacin, the cyclooxygenase blocker, inhibited oedema formation by only 47%. The percent inhibition in oedema formation produced by treatment with 30, 60 and 120 mg/kg, i.p. of AME were 53%, 60% and 77% respectively (Table 2). The degree of reduction in inflammation produced by 30 and 60 mg/kg, i.p. of AME were similar to that produced by indomethacin (Table 2).

### Determination of COX-1 and COX-2 enzyme inhibitory activity in cell free system

The experiments showed that AME does not have any COX-1 inhibitory activity but significantly inhibited the enzymatic activity of COX-2. The percent COX-2 inhibition produced by 5, 10, 15 µg/ml of AME were 50%, 57% and 64% respectively (Table 3).

### Determination of LOX enzyme inhibitory activity in cell free system

AME was found to possess insignificant LOX inhibitory activity.

### Human whole blood assay
### COX-1 assay (determination of thromboxane B2 (TxB2) production)

The percent inhibitions of Thromboxane B2 (TxB2) production in serum samples, as a reflection of COX-1 inhibition, were calculated and insignificant results were obtained.

### COX-2 assay (determination of PGE2 Production)

The percent reduction in PGE2 Production (reflecting the percent COX-2 inhibition) in the 5, 10, 15 µg/ml AME treated plasma samples were 13%, 21% and 30% respectively (Table 3).

### Effect of extract on normal peritoneal cells

AME treatment enhanced the number of macrophages significantly after 6, 12, 24 and 48 h (Table 4). The maximum enhancement in the cell count was observed between 12 - 24 h after AME administration (Table 4). Similar enhancement in the number of normal peritoneal cells was also observed (Data not shown).

## DISCUSSION

The study revealed that the AME of *Swietenia mahagoni* leaves possesses potent anti-inflammatory activity at three different doses of 30, 60 (1/10th of the $LD_{50}$ value) and 120 mg/kg, i.p. in rodents.

**Table 3.** Effect of AME on enzymatic activities of COX-1 and COX-2

| Treatment group | Concentration (mg/ml) | Cell free system % Inhibition | | Human whole blood assay % Inhibition | |
|---|---|---|---|---|---|
| | | COX-1 | COX-2 | COX-1 | COX-2 |
| AME | 5 | NI | 50 ± 0.0365* | NI | 13 ± 0.0035 |
| AME | 10 | NI | 57 ± 0.061* | NI | 21 ± 0.008 |
| AME | 15 | NI | 64 ± 0.048* | NI | 30 ± 0.008* |

NI means no inhibition.
Data are mean ± S.E.M. *denotes significant inhibition as compared to control ($P < 0.05$).

**Table 4.** Effect of AME on macrophage cell count in mice

| Treatment group | Dose (i.p.) | Number of macrophages x 104 | | | |
|---|---|---|---|---|---|
| | | 6 h | 12 h | 24 h | 48 h |
| N.Saline | 0.1 ml/10 g | 122 ± 10.51 | 120 ± 9.87 | 123 ± 10.23 | 119 ± 9.26 |
| AME | 30 mg/kg | 240 ± 23.17* | 240 ± 26.21* | 448 ± 14.66* | 312 ± 16.47* |
| AME | 60 mg/kg | 254 ± 28.27* | 254 ± 25.47* | 686 ± 12.29* | 412 ± 8.0* |
| AME | 120 mg/kg | 400 ± 16.22* | 400 ± 27.40* | 696 ± 19.61* | 424 ± 17.6* |

Data are mean ± S.E.M. *denotes significant inhibition as compared to control ($P < 0.05$).

A series of experiments proved that AME possesses anti-inflammatory activity comparable to that produced by the standard anti-inflammatory drug, ibuprofen, in acute (carrageenan induced paw oedema in rats and croton oil induced ear inflammation in mice), sub-chronic (cotton pellet induced granuloma formation in rats) and chronic (Freund's adjuvant induced polyarthritis in rats) models of inflammation. The finding that AME significantly reduced inflammation in Freund's adjuvant induced polyarthritis in rats shows that AME has antiarthritic activity as well. In vivo experiments showed that the reduction in oedema formation was similar to that of indomethacin in the arachidonic acid induced paw oedema model in rats, indicating that AME might inhibit inflammation by blocking the cyclooxygenase pathway of arachidonic acid metabolism. In vitro studies confirmed the selective cyclooxygenase-2 inhibitory activity of AME. It is interesting to note that in all the models of inflammation AME produced the activity in a dose-dependent manner. Experiments were performed to see the effect of AME on normal murine peritoneal cells. Normally it is found that 50% of the murine intraperitoneal cells are macrophages. Injection of certain materials like casein, Freund's complete adjuvant, thioglycolate, starch, etc. leads to non-specific accumulation of macrophages in the peritoneal cavity. Mature macrophages in the untreated peritoneal cavity are mostly residential. Intraperitoneal administration of different agents results in the exudation and intraperitoneal accumulation of new macrophages, which differ, from the mature macrophages. It has been reported that the exudate macrophages are more active than the residential mature ones in their ability to spread on the surface to which the cells are attached, receptor size of the cell coat, response to chemotactic stimuli, composition of cell wall, etc. (Den Otter *et al.*, 1982). In the present study AME treatment significantly enhanced the peritoneal cell count and also the number of macrophages. Though the actual role of AME in the enhancement of cell count can not

be explained right now, it is possible that the pharmacological effects observed with AME are mediated through changes in the immune system. However, experiments need to be performed to substantiate such a possibility.

It is well known that there is a close relationship between inflammation and cancer (Thun *et al.*, 1991; DuBois, 1995; DuBois *et al.*, 1996). It has been reported that tumor promoters recruit inflammatory cells to the application site and cancer development may also act by aggravating inflammation in the tissue and vice versa and that inflammatory cells are capable of inducing genotoxic effects (Rosin *et al.*, 1994). At the molecular level, products of inflammation, including free radicals, cytokines, growth factors and transcription factors (such as nuclear factor-kappa B) control the expression of cancer genes (e.g., suppressor genes and oncogenes) and key inflammatory enzymes such as cyclooxy-genase-2 (COX-2) and inducible nitric oxide synthase (iNOS). So, it is likely that anti-inflammatory agents may possess anti-cancer activity and vice versa. Since the present study indicates that the extract has significant anti-inflammatory activity, it is plausible that it may possess anti-cancer activity as well. Moreover, suppression of prostaglandin biosynthesis through selective inhibition of cyclooxy-genase (COX)-2 activity is now regarded as an important cancer chemopreventive strategy (Ozaki *et al.*, 1989, Noble, 1990; Chen *et al.*, 2000; Munos *et al.*, 2000) and the present observation of the extract blocking COX-2 pathway of arachidonic acid metabolism provides further support for such a contention that AME may also have anti-cancer properties. The phytochemical analyses performed in the present study demonstrated that alkaloids, saponins and reducing sugars are present in AME. Alkaloids (Wang and Xie, 1999) and saponins (Sparg *et al.*, 2004) have been referenced as anti-inflammatory compounds, a fact, which could explain the anti-inflammatory activity produced by AME.

## ACKNOWLEDGEMENTS

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