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# EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF 75 PERCENT V/V METHANOLIC EXTRACT OF *ABUTILON INDICUM* (LINN.) SWEET LEAVES

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**ABSTRACT**
The active constituent of leaf extract of the plant (*Abutilon indicum*) contains various components such as essential oils in particular eugenol which is reported to possess antioxidant potential. Eugenol is the one of the constituents of plant under investigation. Therefore based on the above facts, it is clear that no scientific studies have been carried out in *Abutilon indicum* regarding the anti-inflammatory and gastro protective activity, the present study has been carried out to investigate and evaluate the anti-inflammatory activity of 75 percent methanolic extract of *Abutilon indicum* (Linn.) Sweet leaves.
**Key words:** Leaves, *Abutilon indicum,* Eugenol, methanolic extract, anti-inflammatory

## INTRODUCTION

A review of literature shows that the following works have been carried out on the plant *Abutilon indicum* (Linn.) Sweet. Especially several pharmacological and phytochemical works have been carried out on the leaves, roots and seeds of *Abutilon indicum* (Linn.) sweet. In particular, phytochemical, pharmacological and pharmacognostical works have been exhaustively done on the leaves of *Abutilon indicum* (Linn.) Sweet. The list of works carried out on the various parts of the plant are as follows.The seeds contain water soluble mucilage and crude protein. It also yields a pale-yellow, semi-drying oil which consists of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid[1,2].The petals contain cyanidin-3-rutinoside, gossypetin-8-glucoside and gossypetin-7-glucoside[3]. Eudesmol, geraniol and caryophyllene identified in essential oil of flowers.Amino acids, glucose, fructose, vitamin-C and galactose, β -pinene, caryophyllene, caryophyllene oxide, cineole, geraniol, geranyl acetate, elemene, eudesmol, farnesol and borneol identified in oil[4]. Fixed oil and gallic acid were isolated from roots[6].Flavonoids have also been isolated[5]. Alkaloids, flavonoids, sterols, saponins, tannins, triterpeniods and glycosides have been identified from different types of extracts of leaves[7].A water soluble galactomannan has been isolated from the seeds containing D-galactose and D-mannose in 2:3 molar ratio. The seed-gum has branched structure consisting of linear chain β-D (1→4) linked mannopyranosyl units, some of which are substituted at 0-6 by two α-D (1→6) galactopyranosyl units mutually linked glycosidically as end groups. An ethanolic extract of the plant showed anticancer and hypothermic activity, and affected the central nervous system in mice[8].Alcoholic and water extracts of *Abutilon indicum* leaves showed significant hypoglycemic effect in normal rats from the 4$^{th}$ hour to 8$^{th}$ hour by approximately 23%[23,24].Antidiarrhoeal activity of leaf extracts of *Abutilon indicum* using petroleum ether, methanol and distilled water was evaluated by gastro intestinal motility, castoroil-induced diarrhoea and prostaglandin $E_2$ – induced enteropooling in rats[9].The aqueous extract of leaves of *Abutilon indicum* was tested for hepatoprotective activity against carbon tetrachloride and paracetamol-induced hepatotoxicities in rats[10]. Preliminary phytochemical tests demonstrated the presence of steroids in petroleum ether and benzene extracts which were found to induce dose dependent CNS depression. Similarly, these extracts showed very good analgesic property, whereas alcoholic and aqueous extracts failed to show analgesic activity but all the extracts of the leaves were found to possess hypoglycemic activity[11].The aqueous extract of the leaves of *Abutilon indicum* showed significant hepato-protective activity against carbon tetrachloride treated rats[12].

### Traditional uses

Leaves are cooked and eaten for bleeding piles. Their extract is used as a diuretic, demulcent and as an emollient fomentation; along with ghee as a remedy for diarrhoea. Decoction of leaves used as mouthwash in toothache and tender gums, also useful in gonorrhoea, inflammation of bladder and for enema and vaginal infection. The leaves are also applied on wounds and ulcers[13].Bark is used as astringent and diuretic[14].Flowers are eaten raw. They are reported to be employed as an application to boils and ulcers. The powdered flowers are eaten with ghee as a remedy in blood vomiting and in cough .Seeds are considered laxative in piles, used in treating cough and are also distinctly useful in gonorrhoea, gleet and chronic cystitis .Root is used as demulcent and diuretic, prescribed in fever, chest infection and urethritis.

## MATERIALS AND METHODS

Fine chemicals used in these experiments were obtained from Sigma Chemical Company, U.S.A. and all other analytical grade chemicals were obtained from S.D. Fine Chemical Ltd., Mumbai.

### Plant materials

The leaves of *Abutilon indicum* (Linn.) Sweet were collected from the region of Chennai, Tamilnadu, in the months of March/April. The plant was identified and authenticated by Research Officer (Pharmacognosy), Central Research Institute (Siddha), Arumbakkam, Chennai-106. A voucher specimen of the plant was deposited at the Department of Pharmacognosy for further reference.

### Extraction and preparation of test sample

The freshly collected leaves were cut into small pieces and 100 gm was soaked in methanol (85% v/v) for 7 days at room temperature with occasional shaking. The container was kept closed throughout the maceration process. At the end of maceration the viscous extract, after filtration was kept in the refrigerator. The extract was then subjected to preliminary qualitative tests to identify the phyto constituents present in the leaf extract. The methanolic extract was administered to the animals by suspending each time in 1% SCMC.

### Experimental animals

Adult wistar rats either sex weighing 180-250 gms were used in the pharmacological and toxicological studies. The inbred animals were taken from the animal house in C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai-96. The animals were maintained in well-ventilated room, temperature was maintained at 22 ± 1$^{o}$C with humidity at 55 ± 5 %. They were fed balanced

rodent pelleted diet from Poultry Research Station, Nandanam, Chennai-35, and tap water *ad libitum* throughout the experimental period. The animals were housed for one week, prior to the experiments to acclimatize to laboratory temperature.The experimental protocol was approved by the Institutional Animal Ethics Committee IAEC Ref No: IAEC XIV-20/CLBMCP/2005-2006.

**Acute oral toxicity study**

The procedure was followed by using OECD 423 guidelines (Organization of Economic Cooperation and Development) (Acute Toxic class method). The acute toxic class method is a stepwise procedure with 3 animals of a single sex per step. Depending on the mortality and/or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data-band scientific conclusion[15].

The method used defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical, which cause acute toxicity.

Six female Wistar rats weighing between 180–250 gm were used for study. The starting dose level of methanolic extract of *Abutilon indicum* (Linn.) Sweet leaf was 2000 mg/kg body weight p.o as most of the crude extracts possess $LD_{50}$ value more than 2000 mg/kg in b.w.p.o. So 2000mg/kg was used as starting dose. Dose was administered to the rats, which were fasted over night with water *ad libitum* and food were withheld for a further 3-4 hours after administration (p.o) of drugs and observed for signs of toxicity. The same dose was once again tried with another three rats and observed for signs of toxicity.

Body weight of the rats before and after treatment were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern were observed and also signs of tremors, convulsions, salivation, diarrhoea, lethargy sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

***In vivo* experimental methods**

**Evaluation of anti-inflammatory activity by carrageenan induced paw oedema model**[16]

Wister rats of either sex weighing 180 to 250 gms were divided into four groups of six animals each.

Group I - Received 1% SCMC 10 ml/kg b.w.(p.o)
Group II - Received MEAI 100 mg/kg b.w (p.o) suspended in 1% SCMC
Group III - Received MEAI 200 mg/kg b.w (p.o) suspended in 1% SCMC
Group IV- Received Indomethacin 10 mg/kg b.w (p.o) suspended in 1% SCMC

The paw edema was induced by injection of 0.1ml of 1% carrageenan in 0.9% saline into sub-plantar region of the left hind paw of the rats. The MEAI, standard (Indomethacin 10 mg/kg) and control (1% SCMC) were administered 60 minutes before carrageenan injection. The volume of injected paw was measured at 1st, 3rd and 5th hour after the carrageenan injection using a plethysmometer and the edema was expressed by increase in paw volume.

The statistical analysis of various studies were carried out using student 't' test and analysis of variance (ANOVA) followed by Dunnett's 't' and Tukeys test, $p < 0.01$ were considered as significant.

**RESULTS AND DISCUSSION**

The acute oral toxicity study was done according to the OECD guidelines 423 (Acute toxic class method) as represented in Table 1.

A starting dose of 2000 mg/kg body weight/p.o of MEAI was administered to 3 female rats, observed for three days. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose level, 2000 mg/kg p.o of methanolic extract of *Abutilon indicum* for 3 days more, and observed for 14 days, no changes were observed from the first set of experiment. $LD_{50}$ cut off mg/kg body weight was observed as X (unclassified) and Globally Harmonized System (GHS) and comes under X (Unclassified).

The maximum effect of edema inhibition was obtained after 1st and 3rd hour of treatment at doses MEAI 100mg/kg and 200mg/kg (47.36%, 42.62 and 50.25%, 66.12% respectively) while standard showed 15.38% and 40.79%, since the standard used was a more powerful anti-inflammatory agent. MEAI showed non-significant activity at a dose as low 100mg/kg b.w of animals. Results are shown in Table 2 and Figure 1.

The mechanism of biological activity of extract *in vivo* may be probably through the following ways: Phospholipid metabolism is catalysed by enzymes such as phospholipase $A_2$ ($PLA_2$), cyclooxygenase ($COX_{1,\ 2\ and\ 3}$), lipooxigenase (LO, 5LO, 12LO and 15LO) and acetyl transferase (AT) that leads to formation of various inflammatory mediators such as prostaglandins (prostanoids) ($PGI_2$, $PGE_{2\alpha}$, $PGD_2$ and $PGE_2$), throboxanes ($TXA_2$), leukotrienes ($LTA_4$, $LTC_4$ and $LTB_4$) and platelet activating factor (PAF).

The phenolic compounds of the extracts, especially flavon-3-ols, flavonols of flavonoids classes, both in free and glycoside form, possess a potent anti-inflammatory activity by targeting COX, LO and AT leading to blockade of their action thereby preventing generation of inflammatory mediators. The enzyme deactivation may be reversible is yet to be known through appropriate experimental model.

It is also probable that unlike conventional NSAIDs the extracts contain several phenolic compounds of diverse chemical structure which may target amino acid domains of the COX by hydrogen bonding to subside the functional status of the enzyme.

**CONCLUSION**

The preliminary phytochemical investigations with methanolic extract of *Abutilon indicum* (Linn.) Sweet leaves showed the presence of terpenes, phenol, essential oil especially eugenol which are confirmed by specific reactions. MEAI was screened for acute toxicity and was found to be non-toxic at the dose level of 200 mg/kg b.w. (p.o.).The anti-inflammatory effect of MEAI was studied by their action on carrageenan – induced paw oedema in albino rats. MEAI produced significant anti-inflammatory activity at early phase. Further studies are being undertaken to isolate and characterise various phytoconstituents and also to establish the influence of the extracts against inflammation.

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Table 1. Acute toxicity class method (OECD guide lines 423) of MEAI

| S. No. | Treatment Group | DOSE | Weight of animal (gm) | | Signs of Toxicity | Onset of Toxicity | Reversible or Irreversible | Duration |
|---|---|---|---|---|---|---|---|---|
| | | | Before Test (on 1st day) | After Test (On 14th day) | | | | |
| 1. | MEAI | 2 gm/kg | 160 | 161 | No sign of toxicity | Nil | Nil | 14 Days |
| 2. | MEAI | 2 gm/kg | 165 | 167 | No sign of toxicity | Nil | Nil | 14 Days |
| 3. | MEAI | 2 gm/kg | 163 | 165 | No sign of toxicity | Nil | Nil | 14 Days |
| As no toxicity or death was observed for the dose level the same dose level was tried again | | | | | | | | |
| 4. | MEAI | 2 gm/kg | 169 | 170 | No sign of toxicity | Nil | Nil | 14 Days |
| 5. | MEAI | 2 gm/kg | 175 | 178 | No sign of toxicity | Nil | Nil | 14 Days |
| 6. | MEAI | 2 gm/kg | 170 | 172 | No sign of toxicity | Nil | Nil | 14 Days |

Table 2. Effect of MEAI on carrageenan induced paw edema

| Group | Treatment | Oedema volume (ml) | | | | |
|---|---|---|---|---|---|---|
| | | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr |
| I | 1% SCMC | 0.193 ± 0.004 | 0.366 ± 0.008 | 0.51 ± 0.013 | 0.506 ± 0.007 | 0.386 ± 0.005 |
| II | MEAI 100 mg/kg, p.o | 0.102 ± 0.006* (47.36) | 0.21 ± 0.008* (42.62) | 0.34 ± 0.003* (33.33) | 0.38 ± 0.013* (24.90) | 0.3167 ± 0.014NS (18.08) |
| III | MEAI 200mg/kg, p.o | 0.096 ± 0.008** (50.25) | 0.124 ± 0.012** (66.12) | 0.28± 0.006* (44.90) | 0.342 ± 0.0134* (32.41) | 0.32 ± 0.015NS (17.09) |
| IV | Indomethacin 20 mg/kg, p.o | 0.163 ± 0.008NS (15.38) | 0.216 ± 0.012* (40.79) | 0.142 ± 0.006** (72.15) | 0.16 ± 0.008** (68.99) | 0.213 ± 0.004* (44.81) |

Values are expressed as mean ± SEM from 6 animals in each group, Percentage inhibition shown in parenthesis, *p<0.01, **p<0.001, NS- Non signifiant.

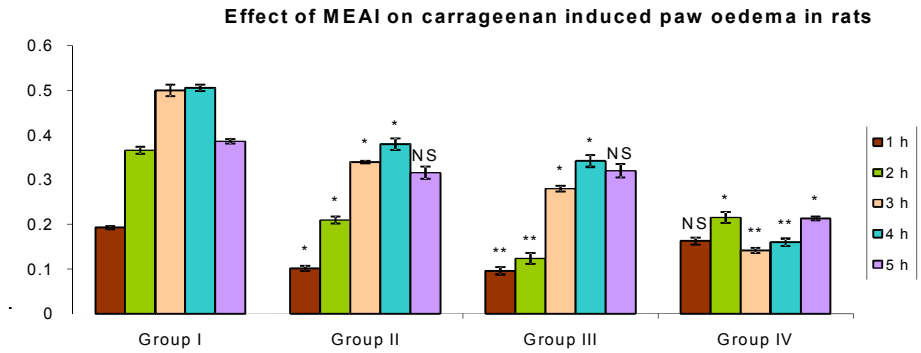


**Figure 1. Effect of MEAI on carrageenan induced paw oedema in rats**

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