



## Evaluation Of Anti-Arthritic Activity Of Methanolic Extract Of *Abutilon Indicum*

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*The various extracts of Abutilon indicum (AI) were investigated for its anti-arthritic activity in In-vitro studies in male albino rats. The evaluation of anti-arthritic activity was carried out using Freund's adjuvant induced arthritis model. Methotrexate (0.75 mg/kg bw) was used as standard drug. The methanolic extract of Abutilon indicum exhibited significant anti-arthritic activity. Treatment with Abutilon indicum 400 mg/kg showed significant reduction ( $P < 0.01$ ) in paw volume on both 7<sup>th</sup> and 14<sup>th</sup> day. Reference standard Methotrexate also showed similar result in this regard. Abutilon indicum 100 and 200 mg/kg were found to be insignificant in reducing paw volume.*

**KEYWORDS:** *Abutilon indicum*, Anti-arthritic, methotrexate, Freund's adjuvant.

### 1.Introduction:

*Abutilon indicum* (Indian *Abutilon*, Indian Mallow; syn. *Sida indica* L.), a traditionally claimed medicinal plant belongs to the family Malvaceae (Chopra *et al*, 1992). It is used traditionally as a laxative and demulcent and for loss of vitality, also to relieve fever through cooling effect and rheumatic pain (Chopra *et al*, 2007; Khare, 2004), febrifuge, anthelmintic, diuretic, antacid, anti – inflammatory especially in urinary and uterine discharges, piles, lumbago (Khare, 2007). It is also recommended as an aphrodisiac, to promote the libido and to relieve menorrhagia (Seetharam *et al*, 2002; Khare, 2004). In addition, Charaka and Sushruta both used this drug as a rasayana with reference to aphrodisiac and revitalising nervine tonic (Khare, 2004). Plant prominently contains mucilage, tannins, aspargines, gallic acid (Kashmiri *et al*, 2009), sesquiterpenes, alkaloids, leucoanthocyanins, flavonoids, sterols, triterpenoids, saponins and cardiac glycosides (Khare, 2007). It has been scientifically documented for its analgesic (Ahmed *et al*, 2000), in vitro anti- inflammatory (Rajurkar *et al*, 2009), antimicrobial *Chromobacterium*, antimycotic (Rajalakshmi and Senthil, 2009), anti oxidant (Kashmiri *et al*, 2009), Hepatoprotective (Sharma *et al*, 1994; Nadeem *et al*, 1997), hypoglycemic (Seetharam *et al*, 2002), anti-diarrhoeal (Chandrashekhar *et al*, 2004), anti-convulsant (Golwala *et al*, 2010), lipid lowering (Pengelly, 2004), wound healing (Roshan *et al*, 2008), anti asthmatic (Paranjape and Mehta, 2008), diuretic (Balamurugan *et al*, 2010) and immunomodulatory activities (Dashputre and Naikwade, 2010).

In succession to a thorough literature review, it is clear that the two widely mentioned claims of this plant i.e. its use in arthritis and libido has not been adequately explored. Hence, it is worthwhile to investigate aerial parts of *Abutilon indicum* for these activities to add scientific data to the current knowledge.

### 2.MATERIALS AND METHODS:

#### 2.1 Plant materials

The Plant material (whole plant) was purchased from respective vendor and was authenticated at official agency. The fresh aerial parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

#### 2.2 Preparation of extracts

The shade-dried aerial plant pulverized to reduce to 60 meshes, powder was charged into Soxhlet apparatus and extraction was carried out using petroleum ether, diethyl ether, chloroform, ethyl acetate and methanol. For water a simple decoction was prepared.

All the extracts were dried by distillation under reduced pressure. Phytochemical analyses of above mentioned different extracts were carried out to test for the presence of various chemical constituents using established methods (Khandelwal, 2006; Kokate, 1997). The extract with presence of maximum number of

phytochemicals of pharmacological importance i.e Methanolic extract of *Abutilon indicum* was selected for further study.

### 2.3 Animals

Wistar albino rats (120-150 gm) and were used. They were maintained at  $25 \pm 2^\circ$  C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle). The animals had free access to food (Amrut feed, Chakan oil mills, India) and water *ad libitum* throughout study. The study was approved by IAEC and all the experiments were carried out between 9:00- 16:00 hour.

### 2.4 Route of Administration

Extracts of *Abutilon indicum* and methotrexate, were administered by oral route using oral feeding needle no 18.

### 2.5 Acute oral toxicity study

Acute toxicity study was performed in healthy albino rats (150-200gm) as per guidelines (AOT 425) suggested by the Organization for Economical Co-operation and Development (OECD). From this data and pilot study reports; three different doses 100, 200 and 400 mg/kg were selected for this study.

## 2.6 Methods for Antiarthritic Activity of AI extract:

### 2.6.1. Complete Freund's adjuvant induced arthritis in rats (Newbould, 1963)

Wistar rats were made arthritic by single intra-dermal injection of 0.1 ml of complete Freund's adjuvant (CFA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into a foot pad of the left hind paw of rats. Paw volume (in ml) was recorded on the 0, 7<sup>th</sup> and the 14<sup>th</sup> day using digital plethysmometer. Thereafter on the 14<sup>th</sup> day radiographic analysis and observation of the secondary lesions was also carried out.

**Table 1: Drug treatment schedule for**

Group	Treatment	Dose
I.	Control (Vehicle)	10 ml/kg p.o.
II.	<i>Abutilon indicum</i> (AI-100)	100mg/kg p.o.
III.	<i>Abutilon indicum</i> (AI-200)	200mg/kg p.o.
IV	<i>Abutilon indicum</i> (AI-400)	400mg/kg p.o.
V.	Methotrexate	0.75 mg/kg p.o.

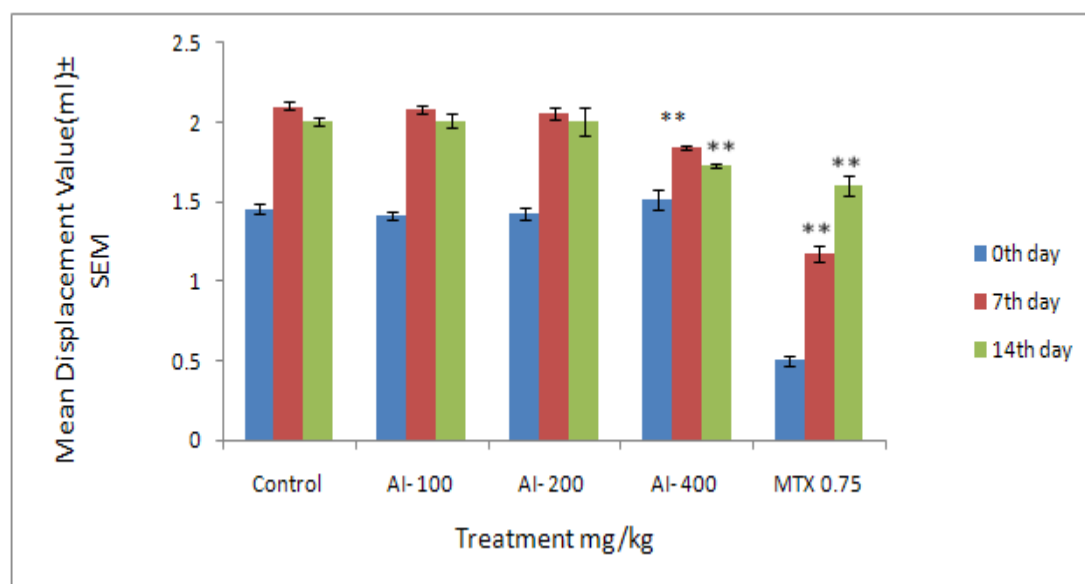
### 2.6.2 Anti-Arthritic Activity

The paw volume displacements of rats pretreated with AI extract 100, 200 and 400 mg/kg, Methotrexate (0.75mg/kg) and control rats on the 0<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> day after CFA injection are given in Table 2. The paw volume displacement showed significant increase on 7<sup>th</sup> and 14<sup>th</sup> day as compared to 0<sup>th</sup> day indicating appearance of inflammation with CFA. Co-treatment with AI 400 mg/kg showed significant reduction ( $P < 0.01$ ) in paw volume on both 7<sup>th</sup> and 14<sup>th</sup> day. Reference standard methotrexate also showed similar result in this regard. AI 100 and 200 mg/kg were found to be insignificant in reducing paw volume.

**Table 2: Effect on paw volume and percentage inhibition of paw volume in Complete Freund’s adjuvant induced arthritic rats.**

Group	Mean Displacement Value(ml)± SEM		
	0 <sup>th</sup>	7 <sup>th</sup> day	14 <sup>th</sup> day
Control (10 ml/kg)	1.456 ± 0.027	2.102 ±0.026	2.005±0.027
AI 100 mg/kg	1.412± 0.021	2.078 ±0.024	2.007±0.045
AI 200 mg/kg	1.426± 0.039	2.056 ±0.042	2.004±0.087
AI 400 mg/kg	1.514± 0.058	1.842 ±0.016**	1.725±0.012**
Methotrexate (0.75 mg/kg)	1.501± 0.028	1.716 ±0.047**	1.601±0.065**

**Figure 1: Effect on paw volume and percentage inhibition of paw volume in Complete Freund’s adjuvant induced arthritic rats.**



**Data Analysis:** Results are expressed as mean ± SEM (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnett’s ‘t’ test. \*P<0.05, \*\*P<0.01.

**Table 3: Effect of AI extract on secondary lesions on 14<sup>th</sup> day.**

No.	Treatment	Secondary lesions
1	Control	+++
2	AI 100 mg/kg	+++
3	AI 200 mg/kg	++
4	AI 400 mg/kg	+
5	Methotrexate	-

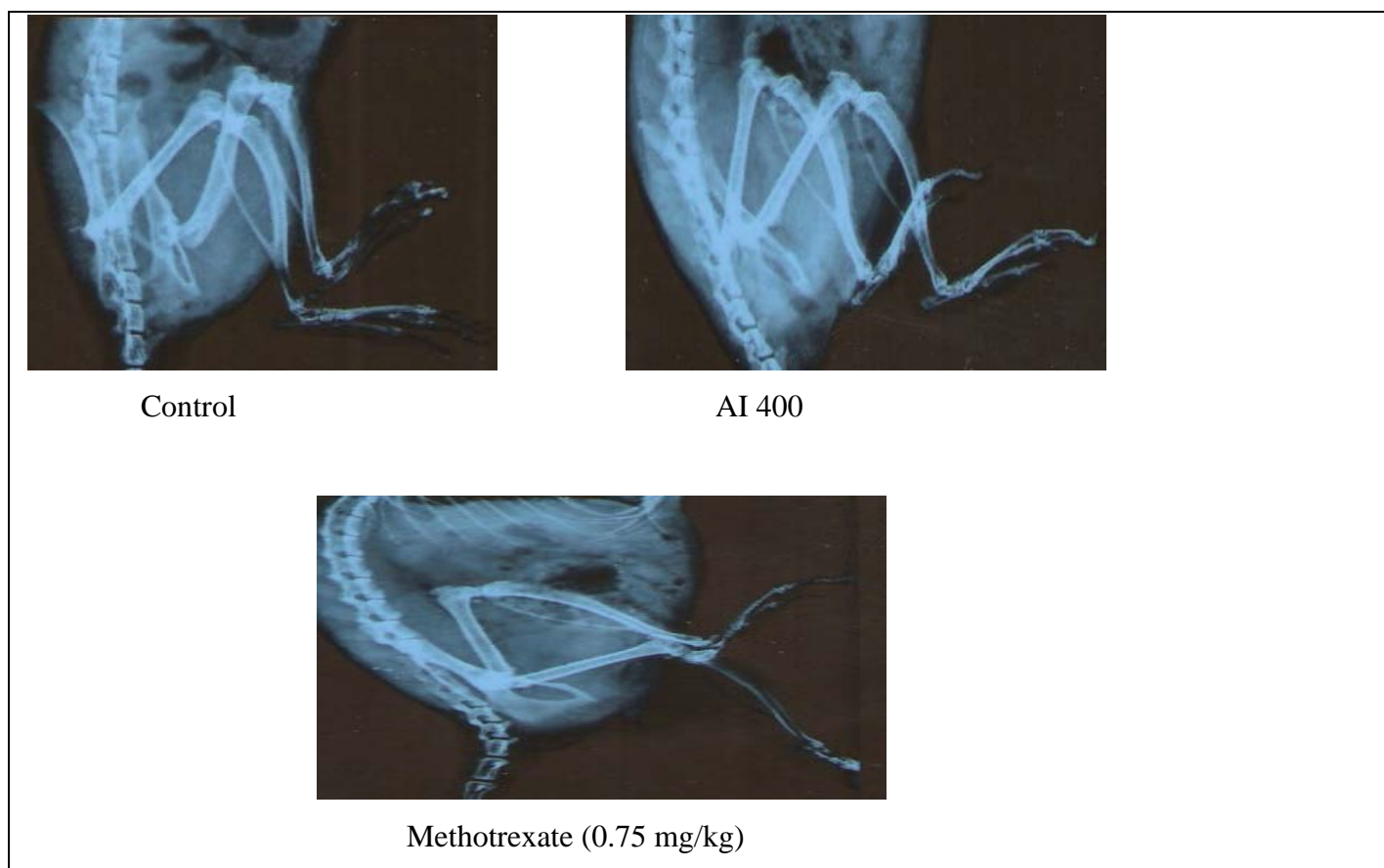
- Nil; + Mild; ++ Moderate; +++ Severe.

*Note: Secondary lesions on the 14<sup>th</sup> day were collectively observed in the ear, fore-paws, hind-paws and tail of rats (Newbould, 1963).*

### 2.6.3 Radiographic analysis

Radiographic examination of FCA injected hind paws of control rat exhibited uneven narrowing of the joint spaces, and subsequent bone cartilage destruction in the knee joint and significant soft tissue swelling indicating full blown arthritis. Whereas AI extracts 400 mg/kg treated rat showed remarkable reduction in soft tissue swelling as well as destruction of the knee joints. Moreover the joint space is more even as compared to the vehicle treated control rats. Similar but more potent results were seen in rats treated with methotrexate.

**Figure 2: Effects of test drug on the proximal interphalangeal joints of control and experimental rats**



### 3. RESULTS AND DISCUSSION:

The present investigation reported that the 400 mg/kg dose of the extract showed significant improvement in arthritic condition by reducing hind paw inflammation and secondary lesions. The improvement in secondary lesions is the hallmark of anti-rheumatoid activity of the extract. These results postulated possible dual role of extract as a symptomatic therapy and preventive remedy which can be considered as a value added outcome as compared to modern therapy.

An ideal therapy in rheumatoid arthritis is expected to halt the disease pathology rather than pure symptomatic relief. Radiographic analysis is considered to be the best tool to screen any drug in this regard. In this study, radiographic analysis of the joint showed significant prevention in the progress of joint pathology which is perhaps the most desired effect in Rheumatoid arthritis. Since Rheumatoid arthritis is an inflammatory immune mediated systemic process of either known or unknown cause (Stobo *et al*, 1996), the number and complexity of clinical manifestation increases with the progress of disease which in turn increases number of drugs especially for symptomatic relief (Beers *et al*, 2008). The common drugs include NSAIDs to relieve pain and inflammation, corticosteroids to control inflammation, immune suppressants to prevent overdue immune response (Beers *et al*, 2008). Most of these drugs are restricted to short term symptomatic relief and are associated with variable side effects that range from gastric ulcers to hepatic damage which adversely affect overall outcome of therapy. In this regard, results of AI are encouraging as it showed all round improvement suggesting possible single substitute which perhaps is the best outcome.

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