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Evaluation of Analgesic and Anti-Inflammatory activity of Abutilon indicum

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Abstract
Most of the synthetic drugs used at present as analgesic and anti-inflammatory agents cause many side effects and toxic effects. Many medicines of plant origin with analgesic and anti-inflammatory activity have been used since long time without adverse effects. The plant Abutilon indicum (AI) is reported to be used as a febrifuge, anthelmintic and anti-inflammatory agent. It is also used to treat ulcers, toothache and hepatic disorders. Thus the present study was undertaken to investigate the analgesic and anti-inflammatory potential of the plant Abutilon indicum. The formalin induced paw licking and tail flick method were used to study the analgesic activity of ethanolic and aqueous extracts of the plant. Carrageenan induced hind paw edema model was used to study anti-inflammatory activity. 200 mg/kg dose was selected to study both activities. Wistar strain albino rats were used for all studies. Diclofenac sodium (5 mg/kg) was used as the standard drug. In tail flick test the increase in the reaction time was highly significant (P < 0.001) with ethanolic and aqueous extracts of the plant Abutilon indicum as compared to the control group. Acute edema in the left hind paw of the animals was induced by sub plantar injection of 0.1 ml (1%) carrageenan suspension in normal saline. The ethanolic extract of the plant significantly (P <0.01) reduced the paw edema in carrageenan treated rats. The effect was maximum at 3hr after the carrageenan injection. The significant suppression of inflammation during the whole experimental period indicates the long duration of action of the ethanolic extract of the plant. Preliminary phytochemical investigation revealed the presence of glycosides, flavonoids, saponins and phenolic compounds in the ethanolic extract of the plant under study. The phytochemical constituents present in these extracts may be responsible for the analgesic and anti-inflammatory activities of the plant Abutilon indicum and the actions may be mediated through CNS and peripheral mechanisms.

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Introduction

Inflammation is a local response of living mammalian tissues to injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. There are various components of an inflammatory reaction that can contribute to the

associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. The drugs which are used presently for the management of pain and inflammatory conditions are either narcotics or non narcotics (NSAIDS), and have known toxic and lethal effects [2]. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to the inhibition of the protective cyclo-oxygenase enzyme in gastric mucosa [3]. On the contrary, herbal medicines with good absorption, less toxicity, and easy availability have been used since ancient times [4]. According to WHO about 80% of the world population still rely mainly on herbal remedies [5-6]. The plant kingdom provides enormous source of pharmacologically active molecules for new drug discovery. Many medicines of plant origin have been used since long time to treat various disorders. It is therefore, essential that efforts be made to introduce new medicinal plants, to develop cheaper, effective and safe analgesic and anti-inflammatory drugs [7].

Abutilon indicum (AI), commonly known as Atibala is a small shrub in the Malvaceae family. In traditional medicine, A. indicum is reported to be used to treat ulcers, headaches, gonorrhea, bladder infection, inflammation and hepatic and pulmonary disorders. It is also used as demulcent, aphrodisiac, laxative, diuretic and sedative (leaves). The bark is astringent and diuretic; laxative, expectorant and demulcent (seeds); anti-inflammatory and anthelmintic (plant); analgesic (fixed oil); diuretic and for leprosy (roots) [8]. The leaves can also be used to treat ulcers, headaches, gonorrhea & bladder infection [9].The plant is very much used in Siddha medicines. In fact the root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men Dr.J.Raamachandran, "Herbs of Siddha Medicines" [10].

A methanol extract of A. indicum had some antimicrobial properties. A chemical compound, β-sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in A. indicum and a petroleum ether extract provided larvicidal properties against the mosquito larvae Culex quinquefasciatus [11].Hence the present study was undertaken to evaluate the therapeutic efficacy of Abutilon indicum in the treatment of peptic ulcer.

Materials and methods

Animals

Male / Female Albino rats of Wistar strain weighing 120–200gm obtained from the Department of Pharmacy, MJRP, were used for the experiment. The animals were housed in cages under standard lab conditions (12:12 hr light / dark cycles at 25±2˚C, RH55±10%). They had free access to standard pellet diet and water ad libitum. The animals were acclimatized at least one week prior to experiment. All experiments were approved by Institutional Animal Ethics Committee (1422/PO/A/11/CPCSEA/PN01).

Plant Materials and Extracts

The whole plant of Abutilon indicum was obtained from Morena, MP. The authenticated plant materials were shade dried and powered coarsely. The coarsely powdered drugs were extracted separately in soxhlet apparatus in sufficient volume of redistilled water and ethanol for 18 hrs. The filtrates were collected and evaporated to dryness on rotary evaporator. The solid masses were collected carefully and weighed. Their yields were calculated and then stored in sealed (air tight) glass bottles at 4˚C for further experimental work.

Treatment protocol

The animals were divided into four groups of six animals each. Group I served as control and received the suspension of 1% CMC in distilled water. Group II received the reference drug diclofenac sodium 5mg/kg. Group III and IV received 200 mg/kg of ethanolic and aqueous extracts of AI by oral route

(p.o.). All the test drugs were suspended in1% CMC and were administered in a volume of 5 ml/kg body weight.

Formalin induced paw licking model

The formalin test comprises the early and late phase assessment of the analgesic effect as described by Hunskaar & Hole [12]. One hour after drug administration by the oral route, 20 µl of 1% formalin was injected subcutaneously sub-plantar in the right hind paw. Then the duration of paw licking as an index of nociception was recorded in periods of 0 to 5 minutes (early phase) and 15 to 30 minutes (late phase) after formalin injection.

Tail flick model

In this model Nichrome wire analgesiometer was used [13]. Individually the tail of each rat was placed over the radiant heat source of the apparatus and the tail withdrawal from the heat (flicking response) was taken as the end point. Analgesic activity was assessed by observing the reaction time in the treated groups. Following the administration of drugs, the reaction time was noted at 0, 30, 60, 90 and 120 min. A cut of time of 15 seconds was considered to avoid tissue injury.

Carrageenan induced paw edema

This test was performed as per the method described by Winter et al [14]. The animals were fasted for 16 hours but water was allowed ad libitum [15]. Acute edema in left hind paws of the rats was induced by the sub plantar injection of 0.1 ml of freshly prepared (1% w/v) carrageenan suspension in normal saline 1h after the drug administration. The paw volume was measured before and 1, 2, 3 and 4 h after the carrageenan injection. The results were expressed as mean ± SEM. The statistical analysis of data was done by the Student's t-test and one-way Analysis of Variance (ANOVA), followed by Dunnet's test. The results were considered statistically significant at $P < 0.05$.

Table No 1: Effect of ethanolic and aqueous extracts of Abutilon indicum on early and late phase of the formalin test

Group	Concentration (mg/kg)	Early phase		Late phase	
		Licking time (s)	% inhibition	Licking time (s)	% inhibition
Control	-	72.33±3.80	-	89.50±7.62	-
Reference Drug (Diclofenac sodium)	5	50.50±4.27**	30.18	35.83±3.37***	59.96
AIE	200	55.00±3.86**	23.95	52.33±5.77**	41.53
AIA	200	61.16±4.26	15.44	59.50±5.21*	34.07

Values are expressed as mean±SEM, n = 6 in each group. *P< 0.05, **P< 0.01, ** *P< 0.001 when compared with control.

Table No 2: Effect of ethanolic and aqueous extracts of Abutilon indicum on Tail flick response in rats

Groups	After 30 min (sec)	After 60 min (sec)	After 90 min (sec)	After 120 min (sec)
Positive control	4.04±0.26	4.02±0.34	4.42±0.32	3.89±0.34
Diclofenac sodium	4.97±0.35	8.11±0.65***	7.19±0.50***	9.33±0.58***
AIE	4.89±0.29	6.65±0.50**	6.96±0.55**	6.96±0.45***
AIA	4.66±0.36	6.25±0.52**	6.26±0.44**	6.49±0.44***

Values are expressed as mean±SEM, n = 6 in each group. *P< 0.05, **P< 0.01, ***P< 0.001 when compared with control.

Table No 3: Effect of ethanolic and aqueous extracts of Abutilon indicum on carrageenan induced paw edema in rats.

Groups	1st hr	2nd hr	3rd hr	4th hr
Positive control	0.62±0.04	0.77±0.05	0.77±0.04	0.67±0.04
Diclofenac sodium	0.53±0.03	0.49±0.04***	0.45±0.03***	0.41±0.03***
AIE	0.56±0.05	0.59±0.03*	0.54±0.04**	0.52±0.03*
AIA	0.56±0.04	0.64±0.04	0.58±0.04*	0.58±0.04

Values are expressed as mean±SEM, n = 6 in each group. *P< 0.05, **P< 0.01, ** *P< 0.001 when compared with control.

Results

Formalin induced paw licking model

The formalin induced paw licking model was used to study the analgesic effects during early and late phase (Table 1). The administration of standard drug diclofenac sodium significantly (P<0.01) inhibited the licking response. The ethanolic extract of Abutilon indicum significantly (P<0.01) inhibited the licking response during the early phase. In the late phase of formalin test, the administration of standard drug highly significantly (P<0.001) inhibited the paw licking response as compared to the control group. The ethanolic and aqueous extracts of Abutilon indicum significantly (P<0.01 and P<0.05 respectively) suppressed the paw licking in the late phase as compared to control group.

Tail flick model

In the tail flick test the ethanolic extract of Abutilon indicum produced a reaction time of 4.89±0.29, 6.65±0.50, 6.96±0.55, 6.96±0.45 seconds after 30, 60, 90 and 120 minutes respectively (Table 2). The reaction time for the same period with control group was found to be 4.04±0.26, 4.02±0.34, 4.42±0.32 and 3.89±0.34 seconds respectively. The reaction time for diclofenac sodium treated group was found to be 4.97±0.35, 8.11±0.65, 7.19±0.50, 9.33±0.58 seconds after 30, 60, 90 and 120 minutes respectively. The reaction time with aqueous extract of Abutilon indicum for the same period was found to be 4.66±0.36, 6.25±0.52, 6.26±0.44 and 6.49±0.44 seconds respectively. The above results show that the increase in reaction time in ethanolic and aqueous extracts treated group was found to be significant after 60, 90 and 120 min (P <0.01, P <0.01 and P <0.001 respectively) as compared to control group. The increase in reaction time in standard drug treated group was found to be highly significant (P <0.001) after 60, 90 and 120 minutes. The increase in reaction time was highest in standard drug treated group and was seen until the last phase observation (120 minutes).

Carrageenan induced paw edema

Development of paw edema was observed in both control and treated groups after carrageenan injection. Thickness of the paw was found to be increased initially upon injection of carrageenan due to volume effect. Difference in the thickness of the rat paw edema was further increased during the time interval of 1h – 4h in control group. When compared with control, the treatment with standard drug significantly suppressed the edema at 2h (P<0.01); and at 3 and 4h (P<0.001). When compared with ethanolic and aqueous extract of AI, the anti- inflammatory activity with the standard drug treatment was more powerful and pronounced. When compared with aqueous extract, the ethanolic extract produced more powerful and pronounced anti-inflammatory activity. The ethanolic extract of AI, significantly reduced the paw edema at 2h (P<0.05), 3h (P<0.01) and 4h (P<0.05). The aqueous extract of AI produced significant suppression (P<0.05) of edema at 3h.

Discussion

In the present investigation anti-inflammatory activity of ethanolic and aqueous extracts of Abutilon Indicum was studied by using inhibition of carrageenan induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. Carrageenan-induced inflammation is a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents [16]. Edema formation in the rat

paw is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins, whereas the second phase is due to the release of prostaglandin and slow reacting substances [17].The ethanolic and aqueous extract of Abutilon indicum significantly reduced the paw edema. In this experiment the suppression of inflammation may be due to PG and kinin synthesis/release inhibition and antihistamine activities. The maximum inflammation is seen approximately three hours post the carrageenan injection, after which it begins to decline.

The formalin model was developed >30 years ago to assess pain and evaluate analgesic drugs in laboratory animals. In this test, a dilute (0.5–5%) formalin solution (in which formaldehyde is the active ingredient) is injected into the paw of a rodent, and pain-related behaviors are assessed over two temporally distinct phases [18-19]. In the present study the analgesic activity of ethanolic and aqueous extracts of Abutilon Indicum was evaluated by formalin induced paw licking and the tail flick model. The persistent pain model of formalin induced hind paw licking was used in the study. The first phase of pain is attributed to the direct activation of nociceptors and primary afferent fibers by formalin, causing the release of bradykinin and trachykinins [20-21]. This phase is inhibited by opioid analgesics. The second phase is due to an inflammatory reaction caused by tissue injury leading to the release of histamine, serotonin, prostaglandin and excitatory amino acids [22-23]. This late phase is inhibited by non-steroidal anti-inflammatory drugs and opioid analgesics

The tail flick model is used to evaluate the analgesic agents acting through central nervous system. In this method a nichrome wire is used to produce pain. In the present study ethanolic and aqueous extracts of Abutilon indicum significantly inhibited the paw licking in the late phase. In the tail flick test the ethanolic extract of AI significantly increased the reaction time. The results of analgesic and anti-inflammatory activities of ethanolic extracts of Abutilon indicum are comparable with standard drug diclofenac sodium.

From the above findings it can be concluded that the ethanolic extract of Abutilon indicum possesses promising analgesic and anti-inflammatory activities. The presence of glycosides, phenolic compounds, carbohydrates, sterols and flavonoids in the ethanolic extract of the plant under study may be responsible for these activities. Further pharmacodynamic investigations are required to understand the precise mechanism of action of Abutilon indicum

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