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Studies on Analgesic and Antiinflammatory Efficacy of *Abutilon indicum*: A Traditional Drug†

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The analgesic and antiinflammatory properties and key chemical constituent from the plant *Abutilon indicum* which is widely used in traditional folk medicine is presented and discussed in the present paper. The analgesic and antiinflammatory potential of plant extracts of *Abutilon indicum* was evaluated scientifically. The analgesic activity was screened by tail immersion method and acetic acid induced writhing method. The antiinflammatory activity was evaluated by formalin induced paw edema method and cotton pellet granuloma method. The antiinflammatory and analgesic potential of methanol extracts were tested at a dose level of 100, 200 and 400 mg/kg body wt. The free radical scavenging activity of the selected plant is also established in chromium induced free radical rat models.

Keywords: *Abutilon indicum*, Analgesic, Antiinflammatory, Antioxidant activity.

INTRODUCTION

The nature has provided a complete ware house of remedies to cure various ailments of mankind. The secrets of Ayurveda individualizing the healing method were preserved in some parts of India. Many medicinal compounds like glycosides, carbohydrates, proteins and amino acids, Saponins, flavanoids, glycosides, phytosterols and phenolic compounds are isolated from these plants. Nature is a best friend of our pharmacy field. Natural drugs are effective in action without side effects. *Abutilon indicum* is an annual prostrate climbing herb found throughout India. It is distributed throughout plains and wetlands. All the parts of plant possess medicinal uses. In folk medicine the plant is reported to be used in treating fever, cough, lung disease, urine output. They are also used in the treatment of deafness, ringing in the ears, high fever, mumps, cough, pulmonary tuberculosis. The whole herb is used in ayurvedic preparations to treat hemorrhoids, diabetes, menorrhoea[1-6]. Leaf extracts of *Abutilon indicum* revealed hypoglycemic activity in experimental rats. But adequate evaluation of its analgesic and antiinflammatory activity has not yet been reported.

EXPERIMENTAL

The plant "*Abutilon indicum*" was collected from in and around Madurai District, Tamil Nadu during the second week of November authenticated by Dr. Stephen, Department of Botany, The American College, Madurai, Tamilnadu, India. The plants were cleaned thoroughly with running water and dried under shade.

Preparation of extracts: Shade dried plants were powdered to get a coarse powder. About 100 g of dry powder was extracted first with the methanol (40-60 ºC) by hot percolation using soxhlet apparatus. The extraction was continued for 72 h. The green residue is obtained. These extracts were used for the study of analgesic and antiinflammatory activities in experimental animals.

Preliminary phytochemical investigation: The qualitative chemical tests were performed for various extracts of *Abutilon indicum* using standard procedure[7-10].

Acute oral toxicity study: Healthy adult male albino rats (150-200 g) were subjected to acute toxicity studies as per OECD guidelines (AOT-425)[11].

Antiinflammatory activity

Formalin induced paw edema method: After 1 h, oral administration of the methanolic extract of *Abutilon indicum* (100, 200 and 400 mg/kg), reference drug (diclofenac sodium, 10 mg/kg), formalin was injected into the right hind limb of each rat under the subplantar aponeurosis. Measurement of paw volume was done by means of volume displacement technique using plethysmometer (Ugo Basile No. 7140)

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immediately after formalin injection and after 1, 2, 3 and 4 h. Percentage inhibition in paw volume was measured and tabulated.

Cotton pellet granuloma method: Cotton pellet induced granuloma method was performed according to the method described by Winter and Porter[12]. Sterile cotton pellets (20 ± 0.5 mg) were implanted subcutaneously in the abdomen region of the rats under anesthesia. The animals received methanolic extract of *Abutilon indicum* (100, 200 and 400 mg/kg), reference drug (Indomethacin, 10 mg/kg) and vehicle (5 % Tween 80) were administered orally, once a day through an oral gavage for seven consecutive days. On the 8th day, the rats were sacrificed, the cotton pellets removed, pellets dried up to constant weight at 60 ºC and the net dry weight was determined.

Analgesic activity

Acetic acid induced writhing test method: The method used in this test has been described by Koster *et al.*[13]. The total number of writhing following intraperitoneal administration of acetic acid solution (1 %, 10 mL/kg) was recorded over a period of 10 min, starting 5 min after acetic acid injection. The mice were administered with the methanolic extract of *Abutilon indicum* (200 and 400 mg/kg), vehicle (5 % tween 80) and standard drug (Aspirin 100 mg/kg). After 0.5 h of drug treatment, 1 % acetic acid is injected peritoneally to induce writhing response. The number of writhing and stretching was recorded and percentage of protection was calculated and tabulated.

Tail immersion test: Tail immersion test was performed according to Aydin *et al.*[14]. The tail up to 3 cm was introduced in warm water maintained at 55 ± 0.5 ºC. Within few minutes, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. The animals were given methanolic extract of *Abutilon indicum* (200 and 400 mg/kg), vehicle (0.5 % Twen 80) and standard drug (Pentazocine, 30 mg/kg), 0.5 h before the immersion of the tail. The time reaction is observed at 1, 2, 3 and 4 after administration of different doses of test drug and standard drug.

Antioxidant studies: Rats were divided randomly into five groups of six animals each and treated for four weeks *i.e.*, 28 days as follows: Group I treated with vehicle at the dose 10 mL/kg. Group II administered with chromium 30 mg/kg (30 % v/v) orally. Group III served as standard group and was administered with vitamin E (200 mg/kg) orally. Group IV and V were treated with methanolic extracts of *Abutilon indicum* at the dose level 200 and 400 mg/kg, respectively. The test substance and standard were administered 1 h prior to chromium(VI) treatment.

Biochemical analysis: Blood was collected on 29th day and serum was separated. Estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were done.

Statistical analysis: Data were analyzed statistically using one way ANOVA followed by Newman Keul's multiple range tests. Probability values less than ($p < 0.01$) were considered significant.

HPLC and LCMSMS analysis: Methanolic extract of *Abutilon indicum* was screened for its active principle using HPLC techniques. 1 mL was pipetted out and diluted to 10 mL with methanol. 20 µL was injected into the HPLC system. LCMSMS analysis was carried out MicrOTOF-QII using CAN and H_2O as mobile phase MSMS fragmentation were carried out in negative ionization mode instrument using protocols given in the instrument manual.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation: Preliminary phyto-chemical screening revealed the presence of alkaloids, saponins, carbohydrates, steroids, glycosides, amino acids, flavonoids, phenolic compounds and tannins.

Acute oral toxicity study: The study revealed that the test drug is non toxic upto a dose level of 2000 mg/Kg bw. The albino rats were observed continuously for behavioral and autonomic profiles for 2 h and for any sign of toxicity or mortality observation were made for 14 days for cyto toxic symptoms. The data on the body weight changes observed were tabulated in Table-1.

TABLE-1
EFFECT OF BODY WEIGHT OF NORMAL AND EXPERIMENTAL ANIMALS IN EACH GROUP

Groups	Initial body weight	Final body weight
Group I	206.6 ± 4.32	212.6 ± 3.30
Group II	200.85 ± 3.87	164.32 ± 2.20*
Group III	210.40 ± 4.32	226.35 ± 5.22*
Group IV	212.75 ± 5.36	230.42 ± 3.68*
Group V	202.76 ± 4.18	226.4 ± 2.20*

Values are represented as mean ± SEM, statistical analysis was performed using one way ANOVA followed by Newman Keul's multiple range test, *Values were significantly different from normal control at $p < 0.01$.

Antiinflammatory activity: The antiinflammatory activity was determined by formalin induced paw edema method by using standard diclofenac sodium and cotton pellet induced granuloma method by using indomethacin as a standard drug. The antiinflammatory activity of methanol extracts at a dose level of 100, 200 and 400 mg/kg body wt were evaluated and presented in Table-2. The antiinflammatory activity evaluated through cotton-pellet granuloma method was given in Table-3.

TABLE-2
EFFECT OF *Abutilon indicum* AGAINST FORMAL IN INDUCED HIND PAW EDEMA METHOD

Group	Treatment (mg/kg)	Initial paw volume (mL)	Paw volume on 7th day (mL)	Inhibition (%)
I	Control	0.681 ± 0.09	0.732 ± 0.10	–
II	Diclofenac sodium (10 mg/kg)	0.562 ± 0.06	0.412 ± 0.04	43.71*
III	M.E.A I (100)	0.612 ± 0.07	0.574 ± 0.06	21.58
IV	M.E.A I (200)	0.564 ± 0.06	0.492 ± 0.09	32.78*
V	M.E.A I (400)	0.542 ± 0.05	0.426 ± 0.08	41.80*

Values are represented as mean ± SEM, statistical analysis was performed using one way ANOVA followed by Newman Keul's multiple range test, *Values were significantly different from normal control at $p < 0.01$.

TABLE-3
EFFECT OF *Abutilon indicum* AGAINST COTTON PELLET INDUCED GRANULOMA METHOD

Group	Treatment (mg/kg)	Weight of granuloma (mg)	Inhibition (%)
I	Control	47.8 ± 2.26	–
II	Indomethacin (10)	20.8 ± 1.13	56.48*
III	Me A I (100)	34.5 ± 1.96	27.82
IV	Me A I (200)	26.75 ± 2.10	44.03*
V	Me A I (400)	22.5 ± 2.01	52.92*

Values are represented as mean ± SEM, statistical analysis was performed using one way ANOVA followed by Newman Keul's multiple range test, *Values were significantly different from normal control at $p < 0.01$.

The antiinflammatory activity and analgesic activity of the test extracts observed were statistically significant.

Analgesic activity: The data obtained on analgesic activity found out by acetic acid induced writhing and tail immersion method were encouraging and presented in Tables 4 and 5, respectively. The analgesic activity of methanol extract was evaluated at a dose level of 200 and 400 mg/kg body wt.

TABLE-4
EFFECT OF *Abutilon indicum* AGAINST ACETIC ACID INDUCED WRITHING METHOD

Group	Treatment (mg/kg)	No of writhing	Inhibition (%)
I	Control	116.25 ± 4.95	–
II	MeAI (200)	88.40 ± 2.65	23.95
III	MeAI (400)	54.31 ± 2.54	53.28*
IV	Aspirin (100)	36.45 ± 1.96	68.64*

Values are represented as mean ± SEM, statistical analysis was performed using one way ANOVA followed by Newman Keul's multiple range test, *Values were significantly different from normal control at $p < 0.01$.

Antioxidant activity: Data presented in Table-6 revealed that the extract possess free radical scavenging effect against chromium induced free radicals in rats. The liver damage in the induced animals is also protected by the test drug treatment which is confirmed through biochemical parameters.

HPLC analysis: HPLC analysis of the bioactive fraction of selected plant drug proved the presence of the flavonoids. Rutin is the only flavonoids with R_f value 0.15 present in a maximum concentration of 4.22 µg/g.

The MSMS fragmentation pattern of rutin obtained through LC-MSMS of the bioactive fraction was presented in Fig. 1. This also confirmed the presence of bioactive molecule rutin detected in HPLC.

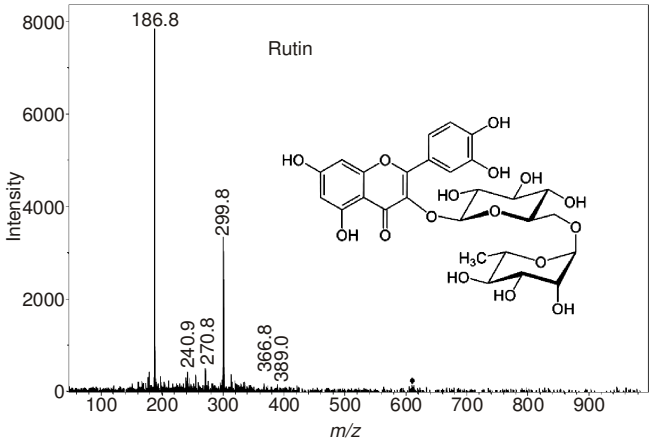

Fig. 1. MSMS Fragmentation pattern of rutin

To sum up, methanol extract of *Abutilon indicum* revealed the presence of flavanoids and significant antioxidant, antiinflammatory and analgesic activities. The test extract was also free from toxicity as observed in acute oral toxicity studies.

Conclusion

The data obtained in the present study depicted that the selected drug *Abutilon indicum* could be a good natural source for developing an antiinflammtory drug with antioxidant and analgesic potentials.

REFERENCES

1. S.N. Yoganarasimhan, Medicinal Plants of India, vol. 2, p. 10 (2000).
2. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, National Institute of Science communication, Council of Scientific and Industrial Research, New Delhi, India (1999).

TABLE-5
EFFECT OF *Abutilon indicum* AGAINST TAIL FLICK LATENCY METHOD

Groups	Treatment (mg/kg)	Dose	Reaction time (mean + SEM)			
			0.5 h	1 h	2 h	3 h
I	Control	10 mL/kg mormal saline	4.60 ± 0.62	4.56 ± 0.60	4.25 ± 0.52	4.09 ± 0.48
II	Me A I	200 mg	7.24 ± 0.96	8.92 ± 1.06	8.49 ± 1.10	7.92 ± 0.96*
III	Me A I	400 mg	6.96 ± 0.88	11.44 ± 1.30	11.62 ± 1.22	10.02 ± 1.12*
IV	Pentazocine	30 mg/kg	7.04 ± 0.90	12.21 ± 1.66	11.46 ± 1.16	12.02 ± 1.36*

Values are represented as mean ± SEM, statistical analysis was performed using one way ANOVA followed by Newman Keul's multiple range test, *aValues were significantly different from normal control at $p < 0.01$.

TABLE-6
EFFECT OF *Abutilon indicum* AGAINST CHROMIUM INDUCED FREE RADICALS IN RATS

Groups	SOD (U/L)	Catalase min/mg of protein	Reduced GSH (mg/dl)	LPO (n mole/mL)	AST (U/L)	ALT (U/L)
Group-I	32.70 ± 1.75	275.8 ± 5.60	115.22 ± 2.80	170.55 ± 2.90	192.85 ± 3.10	88.90 ± 2.72
Group-II	29.38 ± 1.12	187.40 ± 3.38*	61.44 ± 1.35*	330.90 ± 2.72*	330.90 ± 2.72*	222.92 ± 3.15*
Group-III	27.30 ± 1.60	242.85 ± 4.30*	92.4 ± 1.90*	216.8 ± 4.19*	235.30 ± 3.95*	132.55 ± 3.45*
Group-IV	32.12 ± 0.95	208.15 ± 3.50*	80.65 ± 3.20*	201.72 ± 2.45*	264.68 ± 3.25*	155.52 ± 2.48*
Group-V	32.75 ± 1.40	215.48 ± 3.16*	84.30 ± 3.10*	204.50 ± 4.52*	255.51 ± 3.73*	151.72 ± 2.73*

Values are represented as mean ± SEM, statistical analysis was performed using one way ANOVA followed by Newman Keul's multiple range test, *a Values were significantly different from normal control at $p < 0.01$.

3. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Council of Scientific and Industrial Research, New Delhi, Vol 1, p. 21 (1985).
4. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, Vol II, Central Institute of Medicinal and Aromatic Plants, Lucknow (1992).
5. J.B. Harbone, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, New York, edn 2, p. 85 (1984).
6. A. Husain, O.P. Viramani and S.P. Popli, Dictionary of Indian Medicinal Plants, CIMAP, Lucknow, vol. 3 (1992).
7. Trease and Evans, Pharmacognosy, ELBS Publication, Delhi, edn 14, p. 498 (1989).
8. I.L. Finar, Organic Chemistry-Stereochemistry and the Chemistry of Natural Products, Pearson Education Ltd, Singapore, edn 5, p. 518 (1975).
9. H. Plaisted, Philip Contributions from Boyee Thompron Institute, Vol IX, pp. 231-244 (1958).
10. C.K. Kokate, A.R. Purohirt and C.B. Gokhale, Pharmacognosy, Nirali Prakashan, edn 27, p. 344 (2004).
11. M. N. Ghosh, Fundamentals of Experimental Pharmacology, Scientific Book Agency, Kolkatta, edn 2, p. 155 (1984).
12. C.A. Winter an C.C. Porter, *J. Am. Pharm. Assoc. Sci. Educ*., **46**, 515 (1957).
13. R. Koster, M. Anderson, and J. De Beer, Acetic acid for Analgesic Screening, Federation Proceedings, vol. 18, pp. 412-417 (1959).
14. S. Aydin, T. Demir, Y. Ozturk and K.H.C. Baser, *Phytother. Res*., **13**, 20 (1999).