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Analgesic, anti inflammatory and *in-vitro* cytotoxic effects of andrographis paniculata wall. ex Nees

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ABSTRACT

Objective: To evaluate the analgesic, anti-infllammatory and invitro cytotoxic effects of the ethanolic extract of Andrographis paniculata wall ex Nees. **Materials and methods**: The analgesic activity of the ethanolic extract of leaves of Andrographis paniculata (EAP) wall.ex Nees were reported to have analgesic activity but the activity has still not reported in stem. The analgesic activity of Andrographis paniculata was studied in mice by hot plate method, tail clip method and acetic acid induced writhing method. The anti-inflammatory activity of the EAP was studied in rats by carrageenan induced paw edema method. Two different doses of EAP extract (200mg/kg of 400mg/kg body weight) of animals were tested. **Result**: The EAP (400mg/kg) has revealed significant analgesic activity using all the three methods whereas 200mg/kg extract of AP has shown significant activity only using acetic acid induced writhing method. The EAP extract also revealed significant anti-inflammatory activity. EAP was found to be cytotoxic in the vitro model. The invitro cytotoxicity study showed the IC_{50} of EAP to be 62μg/ml. **Conclusion**: The results suggest that the enthanolic extract of AP possesses analgesic, anti-inflammatory and found to be cytotoxic in the *invitro* model.

KEYWORDS

Andrographis paniculata;
Analgesic;
Anti-inflammatory;
EAP.

INTRODUCTION

A large number of Indian Medicinal plants are attributed with various pharmacological activities. Because it containing diversified class of phytochemicals. It is believed that current analgesia-including drug such as opiates and non-steroidal anti-inflammatory drugs are not useful in all cases, because of its side effects and low potency[1]. Most of these medicinal plants have been identified and described by different authors, but efficacy of many of these plants are yet to be scientifically documented. Selection of these plants from the literature were made on the basis of their common use in the treatment of infectious diseases like fever, bronchitis, ulcer, diarrhoea, dysentery and skin diseases[2].

In the present study the medicinal herb[3] Andrographis paniculata wall. ex Nees (Acanthaceae) is commonly found throughout India. It is known as Nilavembu in Tamil, Kirayat in Hindi, Kirata in Sanskrit, Nilaberu in Kannada[4]. The plant is an erect, annual herb growing abundantly in moist, shady grounds upto a height of 0.3-1mm. The major constituents are

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diterpene lactones mainly andrographolide. The herb is reported to possess astringent, anodyne, toxic and alexipharmic properties. The aim of the present study were to evaluate the analgesic, anti- inflammatory and invitro cytotoxic activities of the stem of EAP extract in mice and rats.

MATERIALS AND METHODS

The entire plant was collected from Tajpura, Arcot, Vellore district. The plant was authenticated by Taxanomist, Plant Anatomy Research Centre (PARC), Chennai. The stem was shade dried and ground to obtain course particle size. The powdered material was extracted with 95% ethanol in a continuous hot extractor at 40-50^{0}C. The extract was concentrated and used (5). The percentage yield 8.45% qualitative analysis were performed for the ethanolic extract which showed the presence of diterpene lactones, saponins and flavonoids. Pet ether extract showed the presence of fats and oils.

Animals

Adult male mice (25-30g) were used for all analgesic experiments. Adult male rats (200-250g) were used to study the anti-inflammatory activity. The animals were maintained in colony cages at 25$\pm$2^{0}C, relative humidity 50-55% maintained under 12hr light anddark cycle (6-10hr light, 18-6hr dark). The animals were fed with standard animal feed (Hindustan Lever Ltd) and water was applied adlibitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. The study was conducted after obtaining institutional animal ethical committee clearance (116/June 08/ CPCEA).

Cells

EAC cells were obtained through the courtesy of Amala Cancer Research Center, Thrissur. They were maintained by weekly intra peritoneal inoculation of 10^6cells/mouse.

Acute toxicity study (6)

The study was performed as per the OECD guidelines 423 fifteen male Swiss albino mice of 20-25 gms were used for this study. The extract was prepared in different concentrations (2000, 300, 50, 5 mg/kg p.o) by suitable dilution with tween 20 and water (1:2) and administered orally. Any abnormal clinical signs and mortality of the animal was observed. The ethanolic extract was safe upto a dose of 2000mg/kg body weight so 200mg/kg and 400mg/kg were used as moderate dose for the evaluation.

Evaluation of analgesic activity

Albino male mice were used for the study. The animals were segregated into four groups of six animals each

Group 1-0.5% w/v carboxy methyl cellulose orally; Group 2-Analgin as standard (0.25 ml/animal orally); Group 3-200mg/Kg ethanolic extract orally; Group 4-400mg/Kg ethanolic extract orally.

The dried extract was formulated as a suspension in distilled water.

Hotplate method[7]

The hot-plate (model 7280, Ugo Basile Italy) was maintained at 55^{0}C$\pm$0.2^{0}C and the animals were placed on the surface and the time (sec) to produce discomfort reaction (licking paws or jumping) was recorded. A latency period of 10sec was defined as complete analgesia and the measurement was terminated if it excluded the latency period in order to avoid injury.

Tail-clip method[8]

In this method a bull dog clamp was applied to the base of the tail of the animal and the reaction time was noted in seconds from the time the clip was applied till the animal tried to remove it. A latency period of 10 sec was defined as complete anaIgesia.

Acetic acid induced writhing method[9]

The analgesic activity was assessed using writing test (abdominal constriction test). Acetic acid solution (10ml/Kg, 0.6%) was injected intraperitoneally and the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 0.5h beginning from 5 minutes after acetic acid injection. The extract (200mg and 400mg) was administered 0.5h before the acetic acid injection.

Evaluation of anti inflammatory activity carrageenan induced inflammation

The rats were divided into 4 groups (n=6/group);

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TABLE 1: Qualitative preliminary phytochemical analysis of ethanolic extract of Andrographis paniculata Wall.ex Nees.,

S.no	Substance	Ethanolic extract of stem
1	Saponins	+
2	Proteins	-
3	Tannins	-
4	Steroids	-
5	Glycosides	+
6	Furan	-
7	Phenols	-
8	Falvonoids	+
9	Diterpenoids	+
10	Gum Mucilage	-
11	Alkaloids	-

+ = Present, - = Absent

TABLE 2: Evaluation of analgesic activity of ethanolic extract of andrographis paniculata Wall. ex Nees.,

Method	Control	200 Mg	400Mg	Standard
Hot plate method	4.8± 0.31	6 ± 0.52	8.3±0.67**	8.8±0.75***
Tail clip meyhod	1.7±0.82	4±2.5	8.7±1.2***	9±1.23***
Writing method	30.5±1.18	21.8±0.79***	17±0.73***	18±0.93***

Values are expressed as mean ± SEM (n = 6); **p<0.01, ***p<0.001 as compared to control

TABLE 3: Evaluation of anti-inflammatory activity of ethanolic extract of andrographis paniculata Wall.ex Nees.,

	15 Minutes	30 Minutes	1 hour	2 Hour
Control	1.82±0.07	2.08±0.124	2.01±0.07	2.13±0.08
200Mg	1.52**±0.04	1.70*±0.06	1.71**±0.05	2.08±0 .09
400Mg	1.62±0.06	1.78±0.05	1.67±0.06	1.97±0.08
Standard	1.82±0.03	1.87±0.06	1.83±0.06	2.1±0.11

Values are expressed as mean ± SEM(n=6) *p<0.05, **p< 0.01, as compared to control

TABLE 4: Cytotoxicity of andrographis paniculata Wall.ex Nees., on Ehrilichs ascitis carcinoma by using tryphan blue exclusion

Conc. µg/ ml	Viable cell count	Dead cell count	Total cell count	%cell viable count	% cell death	IC 50.µg/ ml
50	104	76	180	57.7	42.3	
100	194	245	439	44.19	55.81	
200	136	335	471	28.07	71.13	62
400	70	357	427	16.39	83.61	
800	38	475	513	7.4	92.6	
100	8	320	328	2.4	97.6	

Group 1 - 0.5% w/v carboxy methyl cellulose orally; Group 2 - Indomethacin standard (10mg/Kg) orally; Group 3 - 200mg/Kg ethanolic extract orally; Group 4 - 400mg / Kg ethanolic extract orally

The initial hind paw volume of each rat was found by mercury displacement method 0.1 ml of 1% w/v carrageenan was injected after 30 minutes of administration of the control standard and test drug extracts. The paw volume is noted after 15,30,60 and 120 minutes following the administration of carrageenan.

Effect of EAP on *in vitro* cytotoxicity[10]

Short term cytotoxicity was assessed by incubating 1×10^6 EAC cells in 1ml phosphate buffer saline with varying concentratiors of the EAP at 37^0C for 3hrs in CO_2 atmosphere ensured using a Mcintosh Fildes jar. The viability of the cells was determined by the tryphan blue exclusion method.

Statistical analysis

All values were expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA followed by Dunnet's Multiple comparisons using graph pad in stat3 demo and all the results obtained in the study were compared with the vehicle control group. P values <0.001 were considered statistically significant.

RESULTS

The results showed no clinical signs and mortality of the animal therefore an LD 50 >500 mg/kg body weight may be assued. Phytochemical analysis showed the presence of glycosides, saponins, diterpenes and flavonoids (TABLE 1). In pharmacological screening the stem of EAP showed a significantly potent analgesic activity which is comparable to that of the standard analgin 0.25ml/ Kg body weight (TABLE 2). From this study in hot plate and tail clip method the dose 400mg / Kg was significantly different from the control (p<0.00l) but in writhing method 200mg/Kg and 400mg/ Kg were highly significant as compared to control (p<0.001) (TABLE 2). In anti-inflammatory study, the EAP possesses significant anti- inflammatory activity. At 200mg/Kg concentration, the extract was significantly different from the control but the higher dose 400mg/Kg had mild anti inflammatory activity (p<0.05). The standard indomethacin (10mg/Kg) was found to be similar to that of EAP(200mg / Kg) (p<0.0l) (TABLE 3).

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DISCUSSION

The oral administration of EAP reduced carragenean induc d paw edema significantly. The extract showed significant anti-inflammory activity at both low and high doses when compared to the standard. The hot plate and tail immersion method considered to be selective for opioid like compounds in several animal species. The analgesic and anti-inflammatory activities of various herbs have been closely related to the high content of triterpenes[11]. In invitro cytotoxicity study showed the IC_{50} of EAP to be 62µg/ml. So the preliminary phytochemical screening indicated the presence of triterpene and flavonoids. Flavonoids have been shown to possess antimutagenic and antimalignant effects. The present study reveals that the extract was cytotoxic towards EAC. Further studies to characterise the active principles and elucidate the mechanism of the action of EAP are in progress.

REFERENCES

[1] A.Ahmadiani, M.Fereidoni, S.Semnanian; J. Ethnopharmacology, **61**, 229-235 **(1998)**.

[2] S.G.Joshi; Medicinal Plants, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, **(2000)**.

[3] The Wealth of India, Raw Mateials, A Revised Edition, Council of Scientific and Industrial Research, New Delhi, **1**, 264-66.

[4] K.S.Murugesamudhaliyar; Gunapadam, Siddha Materiamedica, 579-80 **(1958)**.

[5] S.V.Gothoskar, K.J.Ranadive; Indian J.Exp.Biol., **9**, 372-5 **(1971)**.

[6] D.J.Ecobichon; 'The Basis of Toxicology Testing', 2nd Edn., New York, CRC Press, 43-60 **(1957)**.

[7] K.A.O'Neil, C.Courtney, R.Rankin, Weissman; J.Pharmacological Methods, **10**, 13-18 **(1983)**.

[8] P.Janssen, C.J.E.Neimemegeers, J.G.H.Dony; Arzneimitted Forschung, **13**, 502-507 **(1963)**.

[9] L.A.Dykstra, J.H.Woods; Journal of Pharmacological Methods, **15**, 263-269 **(1986)**.

[10] K.R.Sheeja, G.Kuttan, R.Kuttan; Amala Res.Bull., **1**, 625-9 **(1997)**.

[11] T.Fotsis, M.S.Pepper, E.Aktas, S.Breit, S.Rosku, H.Adlercreutz; Cancer Res., **57**, 2916-21 **(1997)**.