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Pharmacological potential of *Andrographis paniculata* extracts

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Abstract

Plants have played an important role in human health care since the ancient times and paved path for the discovery of novel drugs. In the present study the pharmacological activities (anti-inflammatory, anti-pyretic and anti-analgesic activity) of chloroform, methanol and aqueous extracts of *Andrographis paniculata* was evaluated to emphasize the potential of herbal components in the field of medical science to cure various dreadful diseases. In order to assess anti-analgesic, anti-pyretic and anti-inflammatory activity, hot water induced analgesic, yeast induced pyrexia and carageenan induced paw oedema was used respectively in rat. In all cases 200mg/kg.p.o of *A. paniculata* extracts were administrated to the Wistar albino rats and the results were compared with control (1ml/kg normal saline). Statistically significant results were obtained in anti-analgesic ($P<0.05$), anti-inflammatory ($P<0.001$) and anti-pyretic activity of chloroform, methanol and aqueous extract of *A. paniculata*. Hence, this study emphasized the potential pharmacological activity of chloroform, methanol and aqueous extracts of *A. paniculata*.

Keywords: *andrographis paniculata*, pharmacological activity, anti-analgesic activity, anti-pyretic activity, anti-inflammatory activity, plant

1. Introduction

Nature has been a potential source of medicinal agents for thousands of years and a huge number of modern drugs have been isolated from natural resources. Medicinal plants have been used in traditional system of medicine, folk medicine, modern medicine, pharmaceutical intermediates, nutraceuticals, chemical entities and food supplements for the synthesis of novel synthetic drugs to control diseases [1]. Plant originated phytomedicines are gaining success due to their cost effectiveness, easy availability and cheaper source for treatment [2]. In recent decades, studies have been carried out on different plant species to discover bioactive compounds of possible interest for medicinal application against diseases. Synthetic drugs that are currently used for the management of pain and for inflammatory conditions cause potential toxic effects [3]. Large numbers of herbal species have been used traditionally or as folk medicines against inflammatory, analgesic and pyretic disorders [4]. In this context *Andrographis paniculata* has been most widely used as a medicinal herb for centuries in several traditional systems of medicine all over the world especially in Indian traditional system and also in tribal medicine.

A.paniculata, commonly known as 'King of Bitter, is a small, annual, branched and erect plant belongs to the family Acanthaceae. It grows abundantly in south eastern Asia including India, Srilanka, Java, Pakistan, Indonesia and Malaysia. It prefers to grow well in a diversity of habitats such as moist, shady areas, hill slopes, plains, farms, seashores, waste lands and dry or wet lands [5]. It is rich in a wide variety of phytochemical constituents such as diterpenes, flavonoids and lactones [6].

A. paniculata is potentially used for the treatment of several varieties of chronic and infectious diseases like gastrointestinal and upper respiratory infections, sore throat, fever, hepatitis, herpes and also dispels toxins from the body [7, 8] and as a cure for dysentery, cholera, influenza, bronchitis, swellings, itheches, piles and gonorrhea [5]. The bioactive compounds isolated from the herbs are andrographolide, neoandrographolide, isoandrographolide, deoxyandrographolide, are reported to possess anti-inflammatory, antianalgesic, antipyretic [9], immunomodulatory [10], antibacterial [11] and anti-HIV activity [12]. The mechanism of action behind the therapeutic value of *A. paniculata* is enzyme induction and the presence of bio active compounds. Hence, the present study is attempted to evaluate the anti-bacterial, anti-inflammatory, anti-analgesic and anti-pyretic activity of chloroform, methanol and aqueous extracts of *A. paniculata*.

2. Methodology

2.1 Collection of *A. paniculata*

The experimental plant species, *A. paniculata* was purchased from the local herbal market. The plant was authenticated and the voucher specimen (Specimen No. JACZOO IM1) was deposited in the herbarium of PG & Research Centre of Zoology, Jayaraj Annapackiam College for Women (A), Periyakulam, S. India.

2.2 Preparation of plant powder

Fresh *A. paniculata* plants were washed thoroughly in tap water followed by distilled water and were then shade dried until all the water content was lost completely. Dried plants were crushed and powdered using blender. Fine powder was obtained after sieving and stored in airtight container until further use.

2.3 Preparation of experimental plant extracts

The plant powder was extracted with three different solvents with an increasing polarity (chloroform, methanol and aqueous solution). The successive extraction was done by a cold maceration process for seven days with regular agitation [13, 14]. After seven days of cold maceration process it was filtered through sterile muslin cloth and the solvent was evaporated using soxhlet apparatus. The residues obtained after evaporation were stored at -20°C until used for experimentation.

2.4 Selection and maintenance of experimental animal

Adult Wistar rats of either sex weighing between 120 - 180gms maintained in Sankaralingam Bhuvaneswari College of Pharmacology Animal House, Sivakasi, Tamil Nadu, S. India were used for the study. The selected animals were housed under standard environmental conditions (temperature 22 ± 1°C; relative humidity 60 ± 5%), maintained in an alternating 12 hr light and dark cycle and had free access to uniform pellet diet and water *ad libitum*. Prior approval of Institutional Animal Ethics Committee (IAEC) was obtained.

2.5 Anti - inflammatory activity

Anti - inflammatory activity was studied following the method described by Winters *et al.* (1962) [15]. Carrageenan was used as a phylogestic agent. Twenty Wistar albino rats of both sex were randomly divided into five groups and each group consisted of four rats weighing between 120 - 180gms. The animals were starved overnight throughout the experiment but enough water was given. Before the administration of experimental drug the volume of paw was measured. Control group received normal saline (1ml/kg), the standard group received a commercially available antianalgesic drug diclofenac sodium (100mg/kg b.w) and the experimental animal groups received 200 mg/kg of chloroform, methanol and aqueous extracts of *A. paniculata*, 30 minutes prior to the injection of carrageenan (0.1ml of 1% w/v solution in normal saline) into sub planter region of left hind paw of each rat. The degree of oedema formation at the hind paw volume was measured using plethysmometer (UGO, Basile) at 0, 1, 2, 3 and 4 hours after carrageenan administration. The inhibition of oedema was calculated in percentage by the following formula.

Percentage inhibition of oedema = A – B = C/A X100.

Where

A= Average increase in paw volume of control

B = Average increase in paw volume after the administration of drug.

2.6 Anti-analgesic activity

Adult Wistar albino rats were screened for its sensitivity by placing the tip of the tail (last 1-2 cm) gently in warm water maintained at 55°C ± 0.5°C. Any rats flicking the tail within 5 sec were selected for the study. The selected rats were divided in to five groups of four animals each. The control and standard groups received distilled water (1ml/kg) and Pentazocine (4 mg/kg p.o) respectively. Other three groups received 200mg/kg p.o of chloroform, methanol and aqueous extracts of *A.paniculata*. The basal reaction time was recorded in all the groups of animals at different time intervals such as 1, 2, 3 and 4 hours after drug treatment [16].

2.7 Anti-pyretic activity

Albino rats of both sex having body temperature between 36.5°C and 38.5°C were selected for the study. The experimental animals were divided into five groups of four animals each. All the rats were injected subcutaneously with 20% aqueous suspension of Brewer's yeast (20ml/kg). The animal developing 0.5°C and more rise in rectal temperature 18 hours after the injection of yeast was selected for further studies. The control and standard groups received distilled water (1ml/kg) and Paracetamol (100 mg/kg p.o) respectively. Other three experimental groups received 200mg/kg p.o of chloroform, methanol and aqueous extracts of *A. paniculata*. The rectal temperature was measured at 0, 1, 2, 3 and 4 hours after the experimental drug treatment [17].

2.7 Statistical analysis

All the data were expressed as mean ± SE. Statistical significance between control and treated groups were assessed by ANOVA followed by Tukey comparison of control and treated groups.

3. Results

3.1 Anti-inflammatory activity

In the present experiment, carrageenan induced anti – inflammatory activity of chloroform, methanol and aqueous extracts of *A. paniculata* was studied in Wistar albino rats. The result revealed that the chloroform, methanol and aqueous extracts of *A. paniculata* significantly (P<0.001) induced the anti-inflammatory activity in terms of inhibition of paw oedema measured at 2, 3 & 4 hrs of post administration compared to control (Table:1).

Table 1: Anti-inflammatory activity of chloroform, methanol and aqueous extracts of *A.paniculata* in rat model

Treatments	Drug & Dose (mg/kg, p.o)	Volume of paw oedema (mm) after drug administration mean ± SE					
		0hrs	1hr	2hrs	3hrs	4hrs	% of oedema
Control	Normal saline (1ml)	0.165 ± 0.018	0.185 ± 0.0180	0.195 ± 0.018	0.2025 ± 0.0125	0.2175 ± 0.008	-
Standard	100 (Diclofenac Sodium)	0.19 ± 0.005	0.175 ± 0.006	0.1025 ± 0.008	0.0675 ± 0.006	0.035 ± 0.006	37.7
Chloroform Extract	200	0.19 ± 0.006	0.2075 ±0.007	0.15 ±0.007	0.085 ± 0.002 ***	0.0425 ± 0.006 ***	26.8
Methanol Extract	200	0.1975 ±0.0075	0.2 ±0.007	0.1525 ± 0.0125	0.08 ± 0.0108 ***	0.055 ± 0.006 ***	25.8
Aqueous Extract	200	0.2 ±0.015	0.22 ± 0.008	0.15 ±0.009	0.0975 ±0.008 ***	0.0425 ± 0.004 ***	23.1

All values are expressed as mean ± SE, n=4; ***P< 0.001 compared to control.

3.2 Anti-analgesic activity

The anti-analgesic activity of chloroform, methanol and aqueous extracts of *A.paniculata* was studied in rat model using tail flick method. According to the result in table 2, all the three extracts of *A.paniculata* positively enhanced the antianalgesic activity at 2, 3 and 4 hrs after drug administration. But statistically significant ($P<0.05$) induction was exhibited by aqueous extract at 4 hrs of post drug administration compared to control.

Table 2: Anti-analgesic effects of chloroform, methanol and aqueous extracts of *A.paniculata* in rat model in terms of animal reaction time to the heat stimulus

Treatments	Drug & Dose (mg/kg, p.o)	After drug administration			
		1hr (sec)	2hrs (sec)	3hrs (sec)	4hrs (sec)
Control	Distilled water (1ml)	3.5 ± 0.288	3.75 ± 0.25	4.75 ± 0.025	3.75 ± 0.25
Standard	4 (Pentazocine)	4.5 ± 0.288	5.75 ± 0.25	6.75 ± 0.25	6.25 ± 0.25
Chloroform Extract	200	2.75 ± 0.25	4.25 ± 0.47	5.75 ± 0.62	5.5 ± 0.64
Methanol Extract	200	2.75 ± 0.47	4.5 ± 0.28	4.75 ± 0.25	4.25 ± 0.25
Aqueous Extract	200	3.5 ± 0.288	4 ± 0.408	5.75 ± 0.25	4.5 ± 0.288 *

All values are expressed as mean ± SE. n=4; *$P<0.05$ compared to control.

3.3 Anti-pyretic activity

In this study, yeast induced anti-pyretic activity was investigated using chloroform, methanol and aqueous extract of *A.paniculata*. Eventhough the results are not statistically significant the chloroform, methanol and aqueous extract decreased the rectal temperature at 2 hrs, 3 hrs and 4 hrs after the drug administration compared to control (table: 3).

Table 3: Anti-pyretic activity of chloroform, methanol and aqueous extracts of *A. paniculata* in rat model.

Treatments	Drug & Dose (mg/kg, p.o)	Rectal temperature (°C) after drug administration (hrs)				
		0hr	1hr	2hrs	3hrs	4hrs
Control	Distilled water (1ml)	37.92 ± 0.35	37.82 ± 0.37	37.85 ± 0.40	37.97 ± 0.38	38 ± 0.37
Standard	100 (Paracetamol)	37.95 ± 0.20	37.5 ± 0.18	37.25 ± 0.18	37.17 ± 0.17	37.05 ± 0.20
Chloroform Extract	200	38.65 ± 0.11	38.02 ±0.08	37.77 ± 0.04	37.7 ± 0.08	37.35 ± 0.06
Methanol Extract	200	38.3 ± 0.10	38.07 ± 0.10	37.82 ± 0.11	37.77 ± 0.09	37.7 ± 0.07
Aqueous Extract	200	38 ± 0.19	37.72 ± 0.14	37.5 ± 0.17	37.32 ± 0.15	37.15 ± 0.10

All values are expressed as mean ± SE. n=4.

4. Discussion

Modern medicine is well developed all over the world, but still large portion of the population in developing countries rely on the traditional practitioners, medicinal plants and herbal medicines for their primary care. Plants have the ability to synthesize a variety of substances which can perform a numerous biological functions and fight against disease causing agents.

In the present study, the *A.paniculata* extracts of chloroform, methanol and aqueous extracts exhibited a significant ($P<0.001$) reduction in carrageenan induced paw oedema, significant ($P<0.05$) decline of hot water induced analgesic activity and inhibition in pyrexia in rats and its effect was compared to the control. Anti-inflammatory activity [18, 19, 20, 21, 22], antianalgesic [23, 9] and antipyretic activities [24, 9] of *A. paniculata* and its phytochemical constituents [25, 26, 27, 28] have been reported by various researchers. Our results are in agreement with the reported literature data. Radhika *et al.* (2009) [29] observed that in 6th hour at a dose of 200mg/kg, the chloroform extract of *A.paniculata* stem showed statistically significant anti-inflammatory activity in rat. The methanol extract of *A.paniculata* exhibited potential anti-analgesic activity followed by the chloroform and petroleum ether extract respectively [30]. The chloroform and aqueous extract (100mg/kg) of *A.paniculata* showed potent anti-pyretic activity when compared to petroleum ether and methanolic extract [31]

The methanol extract of *A.marmelos* exhibited significant anti-inflammatory activity at a dose of 100 mg/kg in albino rats and ethyl acetate extract of *A.monophylla* showed significant anti-inflammatory activity in albino rats [32]. Oral administration of the ethanolic extract (200 and 400 mg/kg, p.o) and its fractions (200mg/kg each) of the aerial parts of *Cleome rutidosperma* produce significant analgesic activity in acetic acid induced writhing and tail immersion tests, anti-inflammatory effect against carrageenin induced inflammation, adjuvant induced polyarthritis and antipyretic activity against yeast induced pyrexia [33].

The core chemical classes of anti-inflammatory agents from natural sources have been reported to engage a huge range of compounds such as polyphenils, flavonoids, terpenoids, alkaloids, anthraquinones, ligans, polysaccharides, saponins and peptides [34]. Terpenoids are an important bioactive compound in plants. It significantly arrests the development of chronic joint swelling and may affect various mechanisms relevant to inflammations arising in response to different etiological factors [35]. Alkaloids in asserted skeletal type based on pyridine ring system have been reported to have enhancing anti – inflammatory activity [36]. Flavonoids have been denoted as nature's biological response modifiers, due to their inherent ability to modify the body's reaction to allergies and virus and they exhibit their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [37] and have been reported as TNF – α inhibitors in different inflammatory condition [38, 39]. *A.paniculata* possesses many secondary metabolites and andrographolide is an important diterpene lactone present in higher amount in leaves and lesser amount in seeds [40]. Roy *et al.* (2010) [41] reported with GC-MS results that phenols, aromatic carboxylic acids and esters present in the chloroform extract of *A.paniculata* to be

responsible for the antimicrobial activity. A diterpene lactone andrographolide, from *A. paniculata* exerts its anti-inflammatory activity by inhibiting NF-KB binding to DNA, and thus reducing the expression of COX-2 [42]. All the above mentioned literature review is in conformation with the results of the present study and revealed that the *A. paniculata* have a potential pharmacological activity.

6. Conclusion

On the basis of the outcome of the present study, it is concluded that, the chloroform, methanol and aqueous extracts of *A.paniculata* are endowed anti-pyretic, anti-analgesic and anti-inflammatory activity. To study the mechanism of action behind the pharmacological activities needs further research and conformations. Our findings and observations offer an alternative medicinal tool to control diseases and provide a scientific support to the ethno medicinal use of the plant. This study confers the use of this plant extract in developing a novel, cost effective and safe broad spectrum of pharmaceutical therapeutic drugs for the betterment of mankind in near future.

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