DRUG DISCOVERY

Effect of *Andrographis paniculata* in experimental models of pain and inflammation

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ABSTRACT

We investigated the effect of a standardized extract of the plant *Andrographis paniculata* in carrageenan induced paw oedema and in acute thermal, electric and visceral inflammatory pain models in rats. The effects of the extract on spontaneous motor activity, haloperidol-induced locomotor impairment and blood glucose level were also examined. Results indicated that *A. paniculata* given at doses of 27, 54 or 108 mg/kg, via the intra peritoneal (i.p.) route caused significant inhibition of the inflammatory paw oedema caused by sub planter injection of carrageenan in a dose dependant manner. In tests of nociception, *A. paniculata* displayed antinociceptive activity in the hot plate, tail electric and acetic acid-induced visceral pain tests. The latency on the hot plate test was delayed by 67.7% and 72.9% while the threshold current required to elicit vocalization in tail electric stimulation test increased by 25% and 30.3% after treatment with *A. paniculata* at 54 and 108 mg/kg, respectively. The above doses of *A. paniculata* caused significant inhibition of the acetic acid-induced writhing by 29.8% and 43.7%, respectively. *A. paniculata* showed no significant effect on spontaneous motor activity or the locomotor impairment caused by haloperidol. It also had no significant effect on blood glucose level in normal rats. Collectively, these results indicated an analgesic an anti-inflammatory activity for *A. paniculata* at doses devoid of a sedative effect.

Keywords: *Andrographis paniculata*, thermal pain, visceral pain, carrageenan, inflammation

1. INTRODUCTION

Andrographis paniculata (Burm.f.) Nees (family *Acanthaceae*) is an annual herbaceous plant, widely grown in Southeast Asia. It used in traditional medicine in China and India as powder, infusion, or decoction either alone or with other medicinal plants for the treatment of various disease aliments including respiratory infections, influenza, dysentery, dyspepsia, malaria and parasitic infections. *Andrographis paniculata* (*A. paniculata)* possesses important pharmacological actions displaying hepatoprotective, anti-inflammatory antipyretic and analgesic actions (Mishra et al., 2007; Hossain et al., 2021). Preparations of standardized *A. paniculata* extracts are commercially available for the treatment of common cold (Hancke et al., 1995; Hu et al., 2017).

A. paniculata extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids and flavonoid glycosides. More than twenty diterpenoids and several flavonoids have been isolated from the plant extracts (Tang and Eisenbrand, 1992). The major diterpenoids in *A. paniculata* are andrographolide, neoandrographolide, 14-deoxyandrographolide, andrographiside, 14-deoxyandrographiside, 14-deoxy-11, 12-didehydroandrographolide and 14-deoxy-11, 12-didehydro-andrographiside (Mishra et al., 2007). The most abundant compound in the leaves is andrographolide. It has been also been extracted from the aerial parts or the whole plant. The flavonoids, apigenin, onsilyin and 3, 4-dicaffeolyquinic are also found (Raman et al., 2022). Andrographolide in particular was endued with anti-inflammatory activity causing the inhibition of inducible nitric oxide synthetase (iNOS) (Chiou et al., 2000), cyclooxygenase-2 (COX-2) expression (Liu et al., 2007) and nuclear factor kappa-B (NF-κB) (Hidalgo et al., 2005; Bao et al, 2009) in inflammatory cells in vitro.

The aim of the present study was to therefore investigate the effects of a commercially available standardized A. *paniculata* extract on acute inflammation caused by subplantar carrageenan and in nociceptive pain models in the rat. In addition, the behavioral effect of *A. paniculata* on locomotor activity and haloperidol-induced motor impairment and blood glucose was studied.

2. MATERIALS AND METHODS

Animals

Experiments were performed using male Sprague–Dawley rats weighing 150–160 g of body weight. Animals were housed under standardized conditions and had free access to food and tap water. Experimental procedures were performed in accordance with the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996). Equal groups of 6 rats/group were used in the experiments.

Drugs and Chemicals

A 4% standardized extract of the plant *Andrographis paniculata* (Remdex: Pharmavite Corp, Northridge, Calif) was used and dissolved in physiological saline to obtain the necessary doses. Carrageenan was purchased from Sigma (St. Louis, U.S.A.). Indomethacin and haloperidol were purchased from Kahira Pharm & Chem. IND Co., Egypt. Haloperidol was dissolved in saline while indomethacin dissolved in 5% sodium bicarbonate solution. Analytical grade glacial acetic acid (Sigma, St. Louis, U.S.A.) is diluted with pyrogen-free physiological saline solution to provide 0.6% solution for the i.p. injection. The doses of *A. paniculata* used in the study were based on the human daily doses after conversion to that of rat and mice using Paget and Barnes conversion tables (1964).

Experimental Methods

Carrageenan-induced paw oedema

Paw swelling was induced by subplantar injection of 1% sterile lambda carrageenan suspension in saline given into the rat right hind paw at a volume of 100 µl (Winter et al., 1962). An equal volume of saline was injected into the left hind paw. *A. paniculata* at doses of 27, 54 or 108 mg/kg or indomethacin at 10 mg/kg was i.p. given 30 min before the injection of carrageenan. The control group received i.p. saline. Quantification of the inflammatory paw oedema was performed by the measurement of the increments in hind footpad thickness with the use of a micrometer caliper just before injection of carrageenan and at 1, 2, 3 and 4h post-carrageenan (Abdel-Salam et al., 2004). Oedema was expressed as the percentage of the basal i.e., pre-saline or pre-drug measurements.

Nociceptive tests

Hot-plate assay

The apparatus used is an electronically controlled hot plate from Ugo Basile, Italy, heated to 53°C (± 0.1°C). Each rat was placed unrestrained on the hot plate for the baseline measurement just prior to the i.p. administration of saline, indomethacin (10 mg/kg) or *A. paniculata* at doses of 27, 54 or 108 mg/kg. Measurements were then taken 1h after saline or drug administration. Latency to lick the hind paw or jump out was recorded for the control and drug-treated groups. The cut-off time was 30s (Le Bars, 2001).

Tail electric stimulation test

Rats (n = 6/group) were i.p. treated with *A. paniculata* (27, 54 or 108 mg/kg) or saline (control) 1h before testing. The minimum current required to elicit vocalization on electrical stimulation of the tail was determined for the control and *A. paniculata*-treated

groups (Michael-Titus and Costentin, 1987). Electrical stimulation of the tail was carried out with Pulse generator 57800-001 (Eugo Basil EXT Unit) (Frequency 50 pulse/sec, shock duration 2 sec).

Acetic acid-induced writhing

Rats were i.p. treated with *A. paniculata* at doses of 27, 54 or 108 mg/kg, indomethacin (10 mg/kg) or saline prior to 0.6% acetic acid injection (0.2 ml, i.p.) (Koster et al., 1959). Drugs were administered 30 min before the induction of the abdominal constriction assay.

Behavioral testing

Spontaneous motor activity

Groups of rats (6/group) were administered either saline or *A. paniculata* at doses of 27, 54 or 108 mg/kg. Thirty min later, each rat was individually placed in an activity monitor that is equipped with photoelectric detectors (Ugo Basile, Italy). The total number of horizontal beam interruptions (spontaneous locomotor activity) was counted over a 6-min period for each animal.

Haloperidol-induced locomotor impairment

Rats (6/group) received i.p. saline, haloperidol (2 mg/kg) or haloperidol in combination with *A. paniculata* at doses of 27, 54 or 108 mg/kg, 30 min prior to testing. Rats then were individually placed in was individually placed in the plastic cage and the number of spontaneous locomotor activity i.e., number of horizontal photobeam breaks was counted over a 6-min time period for each rat.

Blood glucose measurements

Rats (6/group) were i.p. treated with either saline or *A. paniculata* at doses of 27, 54 or 108 mg/kg. After 2h blood samples were withdrawn from the retro-orbital vein plexuses under light ether anaesthesia for blood glucose determination according to the colorimetric method of Tinder, (1969) using spectrophotometer.

Statistical Analysis

Statistical analysis was carried out with the use of one ant two-way analysis of variance (ANOVA) with Duncan's multiple comparisons test for group comparisons. Statistical analysis of results was done using SPSS software (SPSS Inc., Chicago, USA). A probability value less than 0.05 was considered to be statistically significant.

3. RESULTS

Anti-inflammatory activity of *Andrographis paniculata*

The administration of *A. paniculata* inhibited the carrageenan-induced paw oedema response. Two-way ANOVA showed a significant main effect for treatment ($F_{3, 95} = 76.3$; $p = 0.0001$), significant time effect ($F_{3, 95} = 104.2$; $p = 0.0001$) and significant treatment x time interaction ($F_{9, 95} = 36.2$; $p = 0.0001$). Post-hoc analysis revealed significant inhibition of the oedema by all doses of *A. paniculata* at 1, 2, 4 and 4h time points. The percent inhibition of oedema compared to respective control values at 1, 2, 3 and 4h time points were: -44.6, -34, -20.6, -18.3 for 27 mg/kg *A. paniculata,* -45.7, -33.4, -23.4, -21.5% for 54 mg/kg *A. paniculata* and -67.1, -54.8, -45.4, -37.9% for 104 mg/kg *A. paniculata*. Meanwhile, indomethacin inhibited oedema formation by -55.6, -54.3, - 54.4, - 55.9% at 1, 2, 3 and 4h time points, respectively. The group treated with 104 mg/kg of *A. paniculata* exhibited significant suppression of oedema compared to the groups treated with 27 or 54 mg/kg *A. paniculata* at 1, 2, 3 and 4h time points post- carrageenan. On the other hand, indomethacin caused significantly more oedema inhibition compared with 27 or 54 mg/kg *A. paniculata* at 2, 3 or 4h time points and compared with 104 mg/kg *A. paniculata* at 4h time point after carrageenan injection (Figure 1).

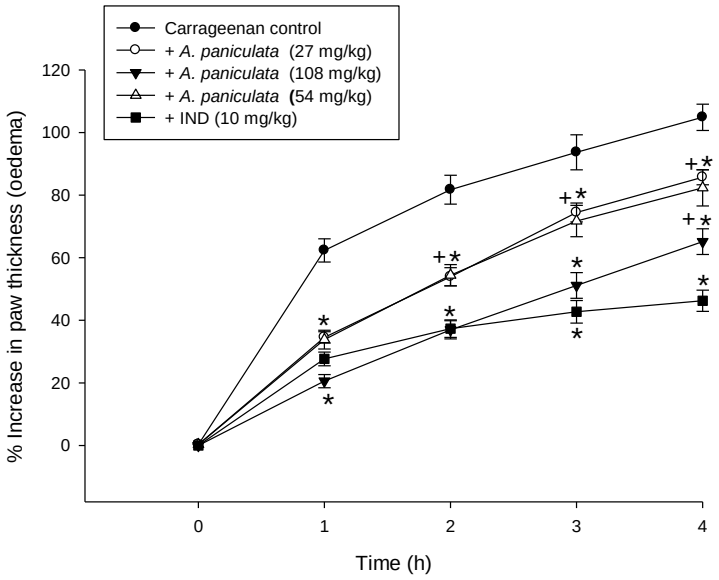

Figure 1 The effect of *A. paniculata* extract and indomethacin (IND) on the paw oedema response to sunplantar injection of carrageenan. Results are expressed as a percentage change from the control (predrug) values. Each point represents mean ± S.E of 6rats/group. *p < 0.05 vs. control value at corresponding time points, +p < 0.05 vs. indomethacin at corresponding time points, Two-way ANOVA and Duncan's multiple comparison test.

Effect of *Andrographis paniculata* in pain models

Hot-plate assay

The reaction time on the hot plate was significantly delayed by *A. paniculata* extract by 44.3%. 67.7% and 72.9% compared with the saline control value, indicating decreased thermal nociception by the extract. Meanwhile, indomethacin given at 10 mg/kg increased hot plate latency by 68.2% compared with the saline control. Values were: 8.8 ± 0.38 sec for the saline group, 12.7 ± 1.1, 14.75 ± 0.79, and 15.22 ± 0.89 sec for *A. paniculata* at doses of 27, 54 or 108 mg/kg, respectively and 14.8 ± 1.36 sec for the indomethacin group (Figure 2).

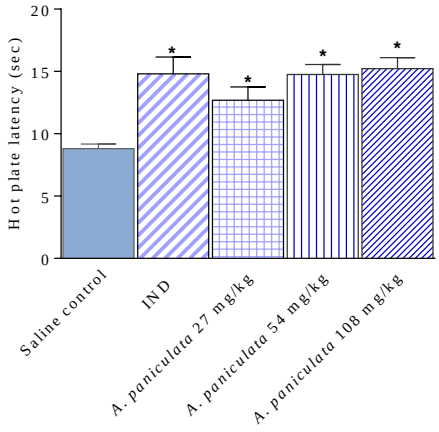

Figure 2 Hot-plate latency measurements for saline, indomethacin (IND) and *A. paniculata* treated rats. Data are mean ± S.E (n = 6/group). *p < 0.05 vs. saline control value. One-way ANOVA and Duncan's multiple comparison test.

Tail electric stimulation test

Treatment with *A. paniculata* extract at doses of 54 or 108 mg/kg caused significant increase in electrical current threshold (μA) in the tail stimulation test by 25% and 30.3%, respectively, compared with the saline control value (275 ± 7.6 and 286.7 ± 13.8 vs. 220 ± 6.8 μA) (Figure 3).

Acetic acid-induced writhing

The visceral nociceptive response to i.p. injection of dilute acetic acid in the rat was significantly inhibited by 29.8% and 43.2% after treatment with A. *paniculata* extract at doses of 54 or 108 mg/kg, respectively (31.8 ± 2.0 and 25.5 ± 1.6 vs. 45.3 ± 1.7). Meanwhile, indomethacin inhibited the writhing response by 55.2% (20.3 ± 1.0 vs. 45.3 ± 1.7) (Figure 4).

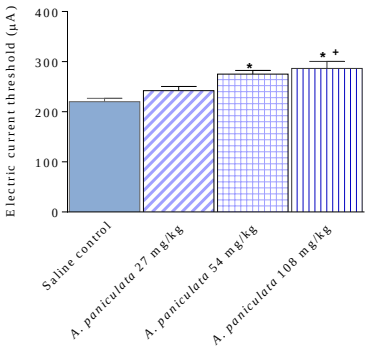

Figure 3 Effect of *A. paniculata* extract on the nociceptive response in the tail electric stimulation in mice. Data are mean ± S.E (n = 6/group). *p < 0.05 vs. saline control value, +p < 0.05 vs. 50 mg/kg *A. paniculata*. One-way ANOVA and Duncan's multiple comparison test.

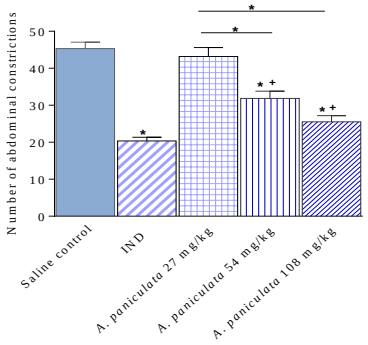

Figure 4 Effect of *A. paniculata* extract or indomethacin (IND) on the number of abdominal constrictions induced by i.p. acetic acid injection. Data are mean ± S.E (n = 6/group). *p < 0.05 vs. saline control value and between different groups as shown in the graph. +p < 0.05 vs. IND group. One-way ANOVA and Duncan's multiple comparison test.

Effect of *Andrographis paniculata* in behavioral tests

Spontaneous motor activity

Compared with the saline control, *A. paniculata* extract had no significant effect on the number of spontaneous movements (Figure 5).

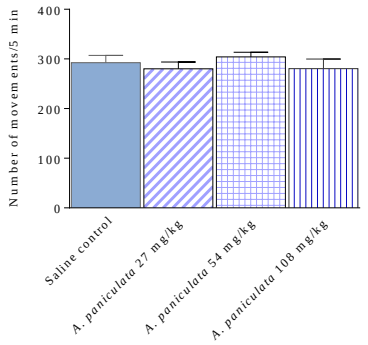

Figure 5 Effect of *A. paniculata* extract spontaneous motor activity. Data are mean ± S.E (n = 6/group).

Haloperidol-induced locomotor impairment

Significant and marked inhibition of spontaneous motor activity was observed in rats treated with haloperidol compared with the saline controls (36 ± 2.7 vs. 285 ± 25.4). *A. paniculata* extract given to haloperidol-treated rats showed no significant effect on the number of spontaneous movements compared with the haloperidol control group (25 ± 1.3, 16 ± 0.81 and 19.3 ± 1.7 vs. 36 ± 2.7) (Figure 6).

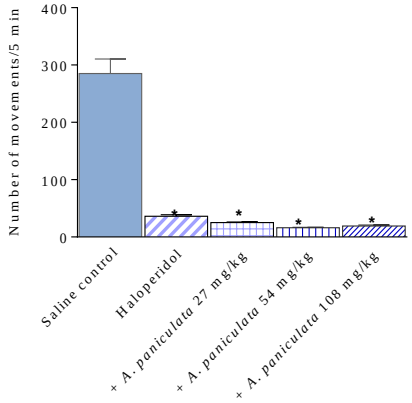

Figure 6 Effect of *A. paniculata* extract on haloperidol-induced motor impairment. Data are mean ± S.E (n = 6/group). *p < 0.05 vs. saline control value. One-way ANOVA and Duncan's multiple comparison test.

Effect of *Andrographis paniculata* on blood glucose

Compared with the saline control, *A. paniculata* extract showed no significant effect on blood glucose level in normal rats. A non-significant decrease in blood glucose by 21.8% was observed with the lower dose of *A. paniculata*. However, a statistically significant difference was observed between 27 mg/kg *A. paniculata* and the higher dose of 108 mg/kg (Figure 7).

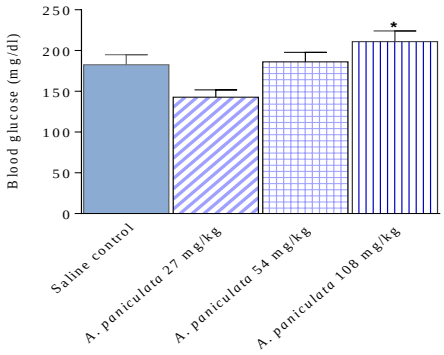

Figure 7 Effect of *A. paniculata* extract on blood glucose in normal fed rats. Data are mean ± S.E (n = 6/group). *p < 0.05 vs. *A. paniculata* 27 mg/kg. One-way ANOVA and Duncan's multiple comparison test.

4. DISCUSSION

Here we investigated the effect of a commercially available preparation of a standardized extract of *A. paniculata* in inflammation and in acute nociceptive pain models in rats. The findings of the present study suggested that *A. paniculata* extract exerts both antiinflammatory and antinociceptive effects. The systemic administration of *A. paniculata* inhibited the development of paw oedema caused by the injection of the inflammogen carrageenan. This antioedema effect of was dose-dependent and more evident in the first hour after inducing inflammation. The latter effect suggests that the extract may be caused by interference with the actions of histamine, serotonin and kinins. These inflammatory mediators have been implicated in the early inflammatory response to carrageenan (Di Rosa et al., 1971).

The effect *A. paniculata* was not significantly different from that obtained with the non-steroidal anti-inflammatory drug (NSAID) indomethacin (10 mg/kg) in the first hour after carrageenan injection. Moreover, the antioedema effect of the higher dose

of the extract didn't differ significantly from that caused by indomethacin at 1, 2 or 3h time points post-carrageenan. An anti-inflammatory activity has also been reported for a chloroform extract of *A. paniculata* stem at 200 mg/kg in the carrageenan model of rat paw oedema (Parvataneni et al., 2009), while neoandrographolide, a diterpene lactone from *A. paniculata* given at 100-150 mg/kg inhibited inflammatory ear edema due to dimethyl benzene in mice (Liu et al., 2007). The effect of *A. paniculata* on inflammation may be ascribed to the active principles like andrographolide and neoandrographolide. These diterpenes have been showed to reduce the expression of cyclooxygenase-2 and inhibit nitric oxide production by inflammatory cells (Hidalgo et al., 2005; Liu et al., 2007).

Our results showed that responses to noxious thermal stimuli on the hot plate test were significantly delayed by *A. paniculata* at all doses examined. The effect of *A. paniculata* was comparable to that of indomethacin. The present study also demonstrated that *A. paniculata* was able to reduce nocifensive behaviors i.e vocalization upon electrical stimulation of the tail. Acute thermal, electrical or chemical noxious stimuli are mediated by cutaneous nociceptors on the peripheral nerve terminals of a subset of peripheral sensory neurons. These nociceptive neurons have unmyelinated C and thinly myelinated Aδ fibers with their cell bodies located in the dorsal root ganglion. Synaptic transmission in the spinal cord is largely mediated by the release of glutamate well as neuropeptides eg., substance P and calcitonin gene-related peptide (Hladnik et al., 2015). The perception of pain is also the subject of descending both facilitatory and inhibitory pathways in the spinal cord (Marshall et al., 2012).

Visceral nociceptive pain can be evoked by i.p. injection of dilute acetic acid in rodents. The determination of the nociceptive response i.e., abdominal constrictions or writhing provides a measure of the severity of pain. This type of inflammatory pain is mediated by the release of prostaglandins, bradykinin and other mediators of inflammation in the peritoneal cavity and these stimulate vagal, spinal or pelvic afferents to transmit nociceptive signals to the central nervous system (Bueno and Fioramonti, 2002; Gebhart and Bielefeldt, 2016). Here, we showed that the administration of *A. paniculata* extract at doses of 54 or 108 mg/kg led to a significant inhibition of the writhing response to i.p. acetic acid injection. The writhing behavior was also markedly inhibited by indomethacin in accordance with studies that indicated the effectiveness of NSAIDs in this model of visceral pain (Santos et al., 1998). *A. paniculata* at doses which were antinociceptive showed not significant effect on spontaneous movement activity or on motor impairment caused by haloperidol administration, which ruled out the influence of possible sedative effect.

Several studies suggested a hypoglycaemic activity for *A. paniculata* extracts in experimentally induced diabetes (Borhanuddin et al., 1994; Zhang and Tan, 2000; Noble et al., 2020). In their study, Zhang and Tan, (2000) using a crude ethanolic extract of *A. paniculata* reported reduction in fasting serum glucose level in diabetic but not in normal rats within 3h after oral administration. However, the increase in serum glucose level in response to glucose load was suppressed by the extract. In rabbits, Borhanuddin et al., (1994) reported a hypoglycaemic activity for a water extract of *A. paniculata (*10 mg/kg) after oral administration of glucose. Meanwhile, there was no significant effect for the extract on fasting blood sugar. The blood sugar lowering effect of *A. paniculata* in experimentally-induced diabetes appears to be mediated by its active principle andrographolide, possibly by increasing glucose ultilization (Yu et al., in 2003). Our results showed that *A. paniculata* extract exerted no significant effect on blood glucose level in normal rats. However, there was a decrease in blood glucose by 21.8%, though non-significant seen only at the lower dose of the extract.

5. CONCLUSIONS

The results of the present study suggest that *A. paniculata* extract may prove of value in the treatment of painful inflammatory conditions.

Author contribution

OMEAS and AAS conducted the research and analysis. OMEAS wrote and prepared the manuscript. OMEAS and AAS approved the final version of the manuscript.

Ethical approval

Experimental procedures were performed in accordance with the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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