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Evaluation of Antipyretic and Anti-inflammatory Activity of Methonolic Extract of *Andrographis paniculata* in Albino Rats

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ABSTRACT

The study was conducted to determine the antipyretic and anti-inflammatory activity of the methanolic extract of *Andographis paniculata* (APE) in albino rats. Acute oral LD50 of APE in female rats was more than 2000 mg/kg. The antipyretic activity was studied by inducing pyrexia with Brewer's yeast. A total number of thirty albino rats (200 g) were used for the study of anti-pyretic activity they were divided into five groups of six rats in each group. Group I served as control Normal saline and Group II were given brewer's yeast alone (20 ml/kg), Group III was administered standard drug Aspirin @ 100 mg /kg body wt. while groups IV and V were treated with 200 mg/kg and 400 mg/kg of *Andrographis paniculata* extract respectively. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's suspension in below the nape of the neck of the animals. The anti-inflammatory activity of APE was assessed by measuring the reduction in carrageenan-induced paw oedema in rats. A twenty four albino rats (200 g) were used for the study of anti-inflammatory activity. Four groups were divided with six rats in each group. Group I served as control Normal saline solution and Group II was administered standard drug phenylbutazone @ 100 mg/kg While, groups III and IV were treated with 200mg/kg and 400mg/kg of *APE* respectively. APE (@ 400 mg/kg had significant antipyretic and anti-inflammatory activity against reduced brewer's yeast induced pyrexia and carrageenan-induced rat paw edema in rats suggesting potent antipyretic effect of APE. From these results it may be concluded that crude methanolic extract of *Andrographis paniculata* have significant antipyretic activity and anti-inflammatory activity that might be due to combined effect of active constituents present in plant extract this strongly support the ethno pharmacological use of the plant for the management of fever and inflammation.

Keywords: Antipyretic activity, methanolic extract, *Andrographis paniculata,* pyrexia.

Pyrexia or fever is a condition where there is abrupt increase in the core temperature above the normal level and it is caused as a secondary impact of infection, tissue damage inflammation, malignancy and other disease states (Chattopadhyay *et al*., 2005; Sharma *et al*., 2010). It is the body's natural defense to create an environment where infectious agents or damaged tissues initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin α, β and TNF-α) which increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (Spacer and Breeder, 1994).

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Most of the antipyretic drugs inhibit cyclooxygenase 2 (COX-2) expressions to reduce elevated body temperature by inhibiting prostaglandin E2 biosynthesis (Chang *et al*., 2005). herbal remedies with antipyretic activity received momentum recently because the synthetic antipyretic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles (Guyon and Hall, 1988). In spite of the newer advancements in synthetic drug development, plants are the major sources for raw materials in drug development. Herbal formulations are not known to cause any notable adverse effects (Kirtikar *et al;* 1984) and are readily available at cheaper prices.

Andrographis paniculata is an herbaceous plant in the Acanthaceae family, commonly known as king of bitter. *Andrographis paniculata* is also known as kalmegh in Ayurvedic herb, medical systems of China and Thailand. In traditional medicine, the aerial parts of the plant particularly the mature leaves are used for treatment of fever, common cold and infectious diseases (Caceres *et al*., 1997; Mishra *et al*., 2007; Valdiani *et al*., 2012). The leaves and root extracts of *A. paniculata* exhibit antimalarial, analgesic , antipyretic and plant is also reported to have immunostimulant property (Lin *et al*., 2009; Shukla *et al.*, 2010; Nety *et al*., 2018)

MATERIALS AND METHODS

Collection of plant material

Certified dried aerial parts powder of *Andrographis Paniculata* were procured from Sanjeevani Ayurved, Chhattishgarh Herbals Quality Forest Products, Durg (C.G).

Preparation of plant extract

The dried powder of aerial parts of *Andrographis Paniculata* were extracted with methanol by using Soxhlet's apparatus at 50-60°C for about 18 hours. About 50 gm of powder was extracted with 250 ml of hot methanol using Soxhlet's apparatus. The extracts were concentrated to dryness in hot water bath to yield crude residue.

Experimental animals

Albino rats (150-200g) of both sexes were obtained from a registered laboratory animal breeder. The animals were housed in polyacrylic cages and maintained in an air conditioned Lab Animal House attached to the Department of Pharmacology and Toxicology. The rats were allowed free access to standard commercial rat pellets All animals were fed with standard laboratory animal diet with free access to clean drinking water. The animals were acclimatized to the laboratory conditions for 10 days before commencement of experiment. All the experimental protocol were approved by the Institutional Animal Ethical Committee (IAEA), College of Veterinary Science & A.H., Anjora, Durg (C.G.), India and were in accordance to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of` Forests and Environment, Government of India.

Acute oral toxicity study

Limit test was performed as per OECD guideline for testing of chemicals (OECD, 1998) to evaluate the acute oral toxicity of APE in female albino rats with the upper limit dose of 2000 mg/kg. The mortality, the behavioral abnormality and signs and symptoms of toxicity, if any, were recorded for a period of 14 days of post administration.

Antipyretic Activity

The antipyretic activity was determined in male Albino rats as per the method described by Loux *et al*. (1972). Thirty adult male Albino rats were randomly assigned to six groups each comprising of six animals (Table 1). The I Group animals served as physiological control where the rats were administered normal saline only. The Group II animals served as pyrexic control group where the rats were administered only with the pyrogen, 20 ml/kg of 20% suspension of Brewer's yeast and at 18 h thereafter the propylene glycol. The Group III rats were treated with Brewer's yeast and at 18 h thereafter the reference antipyretic drug Aspirin 100 mg/kg orally. The rats in Groups IV and V were first administered the Brewer's yeast and at 18 h thereafter they were orally administered with the hot methanolic *Andrographis Paniculata* leaf extract @ 200 mg/kg and 400 mg/kg b wt. respectively.

Table 1: Experimental design for the screening of antipyretic effect of methanolic *A. paniculata* leaf extract (APE) in rats

Group No.	No. of Animals	Treatment
I	6	Physiological Control Normal Saline
II	6	Brewer's yeast (BY)
III	6	Brewer's yeast SC followed by reference drug: Aspirin@ 100 mg/kg, orally after 18h
IV	6	Brewer's yeast SC followed by APE @ 200 mg/kg, orally after 18h
V	6	Brewer's yeast SC followed by APE @ 400 mg/kg, orally after 18h

Anti-inflammatory activity

This test was performed, using rat-paw oedema model induced by phlogestic agent carrageenan, according to the method described by Winter *et al.* (1962). Twenty four male albino rats weighing between 150 -200 gm were kept off feed for 16 hr, but having free access to drinking water were selected for the study. The rats were randomly assigned to four groups, each containing six animals (Table 2). The I Group animals served as control which received normal saline. The II Group rats were orally administered the standard anti-inflammatory drug, phenylbutazone at a dose rate of 100 mg/kg. While group III and group IV animals were treated with 200 mg/kg and 400 mg/kg test APE respectively. The all treatments in the four groups were given one hour before giving the injection of the phlogestic agent into the foot. The phlogestic agent, carrageenan, prepared as 1 per cent suspension in sterile normal saline was injected (0.1 ml) into the planter aponeurosis of right hind paw of the rats with the help of a 26 gauze needle. The volume of the injected- paw of each rat was measured immediately after carrageenan injection (0 h) and subsequently after 3 h. Increase in foot volume 3 h after phlogestic agent was adopted as a measure of the effect and the difference in paw volume (3 h minus 0 h) was adopted as a measure of the inflammatory effect (oedema). The paw volume was measured by water displacement using plethysmometer (Bhat *et al.*, 1977). The percentage of inhibition of oedema was calculated from the difference in oedema volume between treated and control groups. Percentage inhibition in Pedal oedema was calculated as follows:

$$\% \text{ Inhibition} = \frac{A - B}{A \times 100}$$

Where, A = difference in the Paw volume (Pedal oedema) of control group

B = difference in the Paw volume (Pedal oedema) in the treatment group

Table 2: Experimental design for anti-inflammatory study of methanolic *A. paniculata* extract (APE) in albino rats

Group No.	No. of Animals	Treatment
I	6	Control: Normal saline orally and Carrageenan
II	6	Reference Drug: Carrageenan followed by Phenylbutazone @100mg/kg, p.o.
III	6	Carrageenan followed by APE Extract @ 200mg/ kg orally
IV	6	Carrageenan followed by APE Extract @ 400g/Kg orally

Statistical analysis

Data were analyzed by complete randomized design using Analysis of variance Technique and in case of parameters showing significant treatment effect, comparison of mean values were further compared by Duncan's Multiple Range Test (Snedecor and cochran, 1994).

Antipyretic activity of methanolic extracts of *Andrographis Paniculata* was determined in pyrexia induced model with that of the control at different time interval were compared. The antipyretic activity of different concentrations of the extract (200 and 400 mg/kg b wt), standard drug (Aspirin, 100 mg/kg b. wt) and physiological control are depicted in (Table 1, Fig. 1). The results were comparable with standard paracetamol antipyretic effect is dose dependent. Treatment with the extract at dose 200 mg/kg b wt did not produce significant antipyretic activity but the 400 mg/kg b.wt. of the extract and Aspirin (100 mg/kg b wt) significantly decreased the body temperature of the yeast induced pyretic rats after 120 minutes as compared to the control group. The plant extract at a dose of 400 mg/kg b wt showed more potent activity than Aspirin (standard drug).

The initial and final rectal temperature in the group treated with the methanolic extract (200 mg/kg b wt) were 37.72±0.53 and 34.68±0.510C while the initial and final rectal temperature for the Aspirin (100 mg/kg b wt) treated group were 37.14±0.180C and 35.15±0.170C respectively.

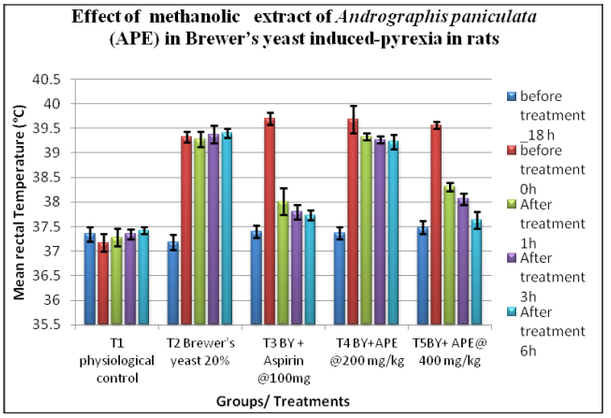

Fig. 1: Effect of methanolic extract of *Andrographis Paniculata* (APE) in Brewer's yeast induced-pyrexia in rats

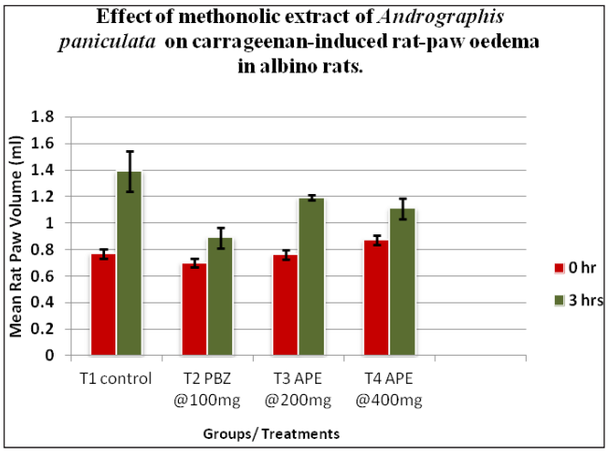

Fig. 2: Effect of supplementation of *Andrographis Paniculata* extract on carrageen an induced rat-paw oedema

The present study reveals that the methanolic extract of *Andrographis paniculata* posses a significant antipyretic effect in the yeast provoked elevation of body temperature in rats and its effect was comparable to that of Paracetamol (standard drug) hence inhibition of prostanglandin synthesis could be possible mechanism of antipyretic action as that of paracetamol (Tjoslen *et al*., 1992). Also there are several mediators for multi processes underlining the pathogenesis of fever, inhibition of any of these mediators may bring about antipyresis (Howard, 1993; Akio *et al.*, 1998; Chandrashekara, 2002; Danquah *et al,* 2011). Further, the methanolic extract was found to contain alkaloids, flavonoids, polyphenolic compounds, tannins terpenes and steroids through preliminary phytochemical screening (Nety *et al.,* 2018). Flavonoids are known to target prostaglandins which are involved in pyrexia. (Raynarayana *et al*., 2006). Hence, the presence of flavonoids and phenol in the methanolic extract of *Andrographis paniculata* may contribute to its antipyretic activity. (Danquah *et al*., 2011).

The anti-inflammatory activity of APE was assessed by measuring the reduction in carrageenan-induced paw oedema in rats. The observations and the results of this study are presented in (Table 2, Fig. 2). The mean edema volumes (ml) of the rats of Group T1 (control), T2 (Phenylbutazone: PBZ; 100mg/kg, p.o.) T3 APE 200 mg/kg and T4 (APE; 400 mg/kg, p.o) were 0.62±0.03 ml, 0.19±0.02 ml, 0.42 and 0.23±0.02 ml, respectively. The results showed that pretreatment of rats with PBZ and APE individually produced significant ($p < 0.001$) reduction of carrageenan induced acute paw edema in rats and the percentages of inhibition of oedema in groups T2, T3 and T4 were 69.35, 32.35 and 62.90, respectively as compared to untreated control. The results indicated that APE had significant anti-inflammatory activity against carrageenan-induced rat paw oedema and the anti-inflammatory effect of APE is comparable to that of the standard drug, phenylbutazone.

The injection of carrageenan into the hind feet of the rats causes local oedema and this process forms the basis of commonly used test for non-steroidal anti-inflammatory drugs (NSAIDs). The early vascular changes and the oedematous phase following disruption of permeability as induced by carrageenan, were effectively antagonized by APE. The mediators in carrageenan induced oedema are histamine, 5-HT, kinins and prostaglandins at various time intervals (Chang *et al.*, 2005). Thus, the anti-inflammatory activity of APE observed in the present study, could be because of the antagonistic action of APE on inflammatory mediators. The results of the present study are in agreement with the results of (Radhika *et al.* 2009) who reported anti-inflammatory activity of chloroform extract of *Andrographis paniculata* using carrageenan

induced rat hind paw oedema model and attributed its anti-inflammatory activity to the presence of andrographolide abundantly available in the leaves of the plant.

CONCLUSION

In conclusion, present findings demonstrated that the crude methanolic extract of *Andrographis paniculata* have significant antipyretic and anti-inflammatory activities when compared with the standard drug, which are involved in possible inhibition of the central synthesis of prostaglandins. However, further studies are necessary to fully elucidate the mechanism of action of plant extract. The maximum significant antipyretic and anti-inflammatory activity that might be due to combined effect of active constituents Andrographolide, Flavonoids and polyphenolic compounds present in plant extract. This strongly supports the ethno pharmacological use of the plant for the management of fever and inflammation.

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