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ANDROGRAPHIS PANICULATA EXTRACT: ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

The *Andrographis paniculata* have been used in traditional folklore for various inflammatory and analgesic uses amongst other uses. In present study we evaluated the analgesic and anti-inflammatory activity of the leaf extract of *Andrographis paniculata*. The extraction of leaves powder was done in three different solvent aqueous, methanol and ethanol. All three extract did not show any acute toxicity. Analgesic effects were tested in mice using hot plate and writhing tests. The results showed that, at 100 mg/kg, all tested substances have significant analgesic effects, and the highest potency was seen with 200 and 300 mg/kg. Anti-inflammatory activity were tested by carrageenan induced paw edema in rats. Methanol extract (200 and 400 mg/kg) showed a significant ($p<0.001$) reduction in paw volume in a dose dependent manner at 3rd and 5th h. The inhibitory effect of the Methanol extract at (400 mg/kg) was found to be 40.92% at 3rd h and 51.47% at 5th. However, aqueous extract (400 mg/kg) showed significant ($p<0.001$) inhibition in paw volume at 5th h with 22.76% inhibition when compared to carrageenan control group. These could be further developed as analgesic, antipyretic and anti-inflammatory agents, without any serious toxicity.

KEYWORDS: *Andrographis paniculata*, Analgesic, Anti-inflammatory, extraction, toxicity.

1. INTRODUCTION

Pain and inflammation are associated with pathophysiology of various diseases like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medicinal systems to relief symptoms of pain and inflammation.[1] Over the centuries number of medicinal plants has been exploited for the treatment of the disorders associated with the inflammatory conditions or for the control of inflammatory aspects of diseases. These medicinal plants owe their activities due to the phyto-constituents and may exert anti-inflammatory effect by interfering generally with the inflammatory pathways or especially with certain components of the pathway, such as release of pro-inflammatory mediators, migration of leukocytes under inflammatory stimulus with consequent release of the cytoplasmic contents at inflammatory sites.[2]

Throughout the evolutions, the importance of natural products for medicine and health has been enormous. Since our earliest ancestors used certain herbs to relieve pain or wrapped leaves around wounds to improve healing, natural products have often been the sole means to treat disease and injuries. In fact, it has been during past decades that natural products taken a secondary role in drug discovery and drug development, after the advent of molecular biology and combinatorial chemistry made possible the rational design of chemical compounds to target specific molecules. The past few years, however have seen a renewed interest in the use of natural products and more importantly their role as a basis for drug development. Numerous useful drugs are developed from lead compounds discovered from medicinal plants. In addition, the elucidation of the molecular structure of many natural products allowed chemists to synthesize them, rather than isolating them from natural sources, which markedly lowered the cost of drug production. Subsequently, a large number of well known natural compounds were identified, analyzed and synthesized. The structural analysis of natural compounds and the ability to synthesize them allowed chemists to modify them in order to suppress or enhance certain characteristics such as solubility, efficiency, or stability in human body. Newman and Cragg (2008) estimated that about 60% of the drugs that are now available such as artemisinin, camptothecin, lovastatin were either directly or indirectly derived from natural products.[3] Moreover, natural products have also been an invaluable source of inspiration for organic chemists to synthesize novel drug candidates.[4]

This is not surprising that medicinal plants have been a part of the tradition and culture of the Asian people from a very long time especially in India. Survey of pharma sector shows that, interest of pharma industry in medicinal plants is increasing day by day. The

Andrographis paniculata for pharmaceutical potential was taken into study so that it turns to be helpful for the pharmaceutical industries and in the long run can eventually benefit the human race. In order to achieve the objectives of the study, various tests were performed, by the help of which potential and medicinal qualities of the plant *Andrographis paniculata* were enormously highlighted.[5]

Andrographis paniculata is reported to contain chemical constituents which may exert analgesic and anti-inflammatory effect.[6,7] Therefore the present investigation was to extract *Andrographis paniculata* leaves and evaluate analgesic and anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh and healthy leaves of *Andrographis paniculata* were collected and washed thoroughly with distilled water and dried in shade for seven days followed by grinding and then coarse powder stored in air tight bottles.

2.2. Extraction of *andrographis paniculata* leaves

The shade dried coarse powder of leaves of *Andrographis paniculata* (50gm) was extracted using 250 ml of the extraction solvent using soxhlet apparatus. The solvents used for the extraction procedure were ethanol, methanol and distilled water. The extracts were concentrated to dryness to yield crude residues. The extracts were auto-claved and stored at 4°C, until further use.

2.3. Quantitative analysis of *Andrographis paniculata* leaves extracts by HPLC

Phytochemical analysis of ethanol 80% (EtOH) and aqueous extracts of *Andrographis paniculata* leaves was carried out using RPHPLC based on modified method described by Xu et al. (2008) for quantification of chemical constituents such as andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide. The ethanol and water extracts were individually prepared by dissolving 10 mg of leaves extracts in 1 ml of HPLC-grade methanol solvent, while the chemical markers solution was made by mixing 200 g of each of the three standard diterpene lactones in 1 ml of HPLC-grade methanol. Extract and standard solutions were filtered through a 0.45 m Millipore Millex PTFE membrane. Separate analyses of the extract and standard solutions were performed under the following conditions; column: reversed phase, C-18 column, detector: PDA (Waters 2998), wavelength: 205 nm, flow rate: 0.8 ml/min, mobile phase: A. methanol, B. water isocratically eluted with 55% A for 30 min with a 10-min equilibration period before injection. The injection volume of the solution was 10 µL.

2.4. Analgesic activity

Following animal models were used for evaluation of analgesic activity:

(1) Hot plate test in mice

The hot plate test that apply heat stimuli to the hind paws are considered to integrate supraspinal pathways, as rats with spinal transection do not withdraw the hind limbs in the hot plate test.[8] Female Swiss albino mice (25 – 30 g) were treated according to the method described by Eddy and Leimback, 1953. Mice were screened by placing them on hot plate maintained at 55 ± 1 °C and the reaction time was recorded in seconds. The time for paw licking or jumping on the hot plate was considered as a reaction time. The responses were recorded before and after 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 hours of the administration of aqueous, methanol, ethanol and pentazocine. A cut-off time of 32 seconds was used to avoid injury to the animals.[9] The percentage protection against thermal pain stimulus was calculated according to the following formula.[10]

$$\text{Percentage protection against thermal stimulus} = \frac{\text{Test mean (Ta)} - \text{Control mean (Tb)}}{\text{Control mean (Tb)}} \times 100.$$

(2) Acetic acid induced writhing in mice

Mice were divided into five groups (n = 8 per group) and administrated with extracct (200, 600, and 1000 mg/kg), control (distilled water), and acetylsalicylic 200 mg/kg. Then, 30 min after oral administration, 0.7% acetic acid was injected in peritoneal cavity at 10 mL/kg, and after 10 min writhing responses were recorded. A twist reaction was composed of a contraction of the abdominal wall and a turn of the pelvis following the swelling of the hind limbs. A significant decrease in writhing response in the administered group compared with the control group was considered as pain response.[11,12]

2.5. Anti-inflammatory activity

Following methods were used for evaluation of anti-inflammatory activity

(A) Carrageenan-induced paw oedema in rats

For *in-vivo* evaluation of anti-inflammatory activity of leaves extract of *Andrographis paniculata* total 5 healthy female Wister Albino rats weight between 120-200 grams was selected. Throughout the experimentation, the animals were individually housed in separate cages within the animal facility, adhering to standard laboratory protocols. The rats were divided into five groups and subjected to overnight fasting, with access restricted solely to water ad libitum. Paw volume of each rat were measure by using a Plethysmometer.[13,14]

Group 1: served as the control and received a normal saline treatment.

Group 2 was administered the standard treatment of Diclofenac Sodium.

Groups 3, 4, and 5 were subjected to doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, of the methanolic extract of leaves of plant.

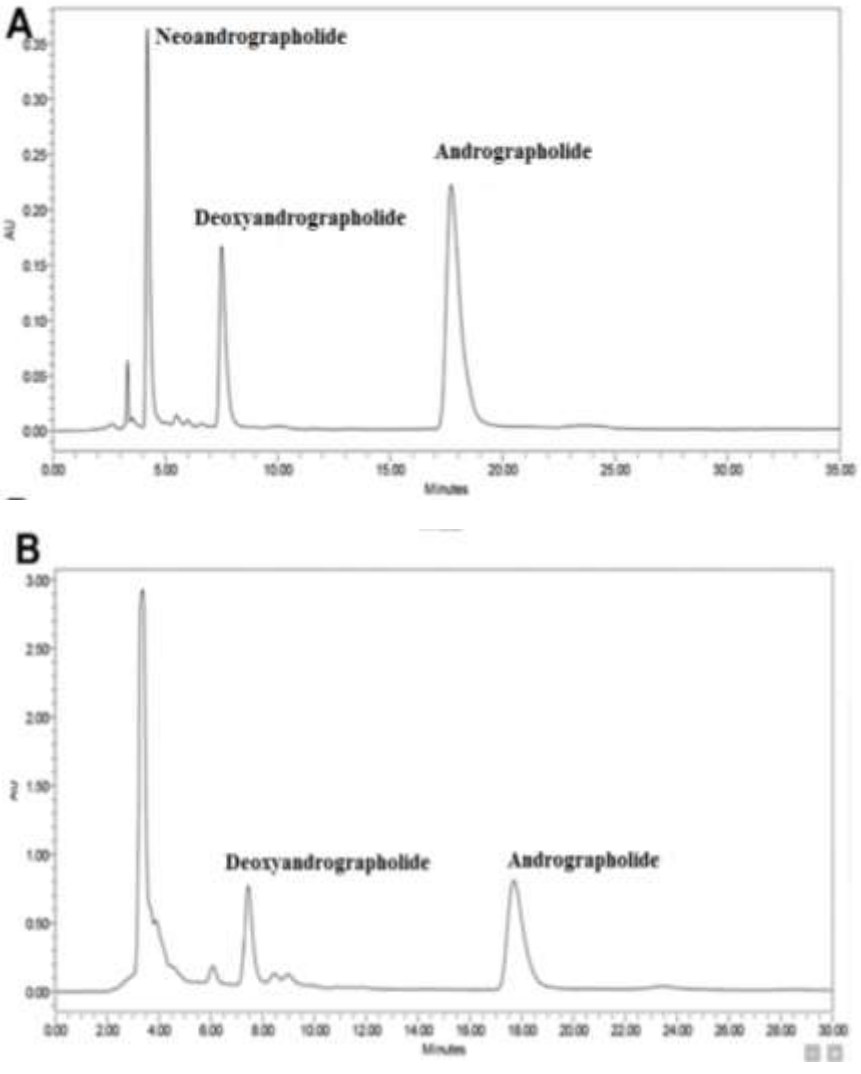
Following group allocation and treatments, 0.1ml of 1% Carrageenan solution was injected into the left hind paw of each albino rat, with the time of injection duly recorded. The extract was orally administered to the rats 30 minutes subsequent to Carrageenan induction. Subsequent paw volume readings were recorded at various time intervals (0 hour, 1 hour, 2 hours, and 3 hours) for each albino rat. The percentage inhibition of each group was determined using the following formula:
% inhibition = (Vc−Vt / Vc)× 100

The data provided in this study are presented as Mean ± SD calculated from individual observations. Statistical analysis for the in-vivo anti-inflammatory activity was performed using one-way ANOVA, with significance indicated by $P < 0.05$.

3. RESULT AND DISCUSSION

3.1. Quantitative analysis of *Andrographis paniculata* leaves extracts by HPLC

Three diterpene lactones andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide were detected in *Andrographis paniculata* leaves extracts by HPLC (Figure 1). Table 1 summarizes the amount of diterpene lactones found in *Andrographis paniculata* leaves. In general, the EtOH extract contained more diterpene lactones than the aqueous extract. andrographolide was identified at a concentration of 100.40 mg/g (10.34% w/w) in EtOH extract, which was greater than the concentrations of the other identifiable phytochemicals.



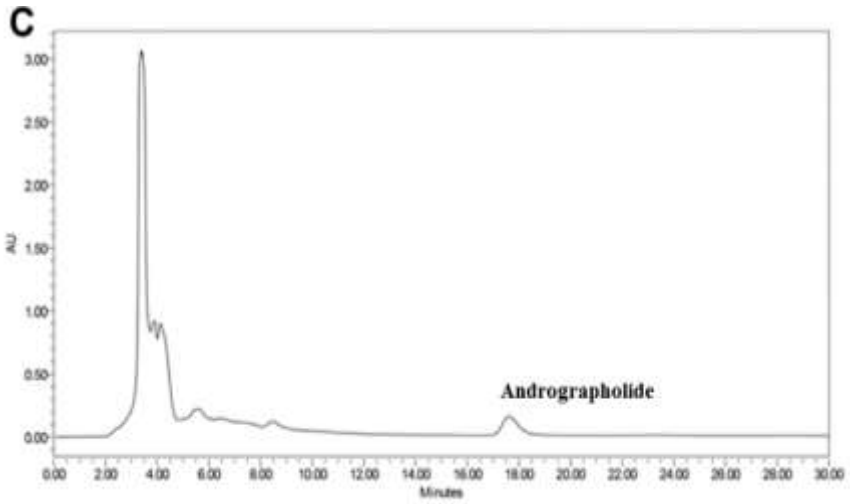


Figure 1: HPLC chromatograms of andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide (A), ethanol extract (B) and water extract (C) of Andrographis paniculata leaves.

Table 1: Amount of diterpene lactones in ethanol and water extracts of *Andrographis paniculata* leaves analyzed by HPLC.

Standard	Concentration (mg/g)		Amount (% w/w)	
	Ethanol	Water	Ethanol	Water
Andrographolide	113.44 ± 1.63	13.788 ± 1.61	11.344 ± 0.16	1.379 ± 0.16
14-deoxy-11,12-didehydroandrographolide	74.350 ± 1.88	ND	7.435 ± 0.19	ND
Neoandrographolide	ND	ND	ND	ND

3.2. Analgesic activity

(a) Hot plate test in mice

The results of the analgesic activity of *Andrographis paniculata* leaves extract using hot plate method are presented in Table 2. There was no significant difference on the thermal stimulus in mice treated with the vehicle (negative control) throughout the whole time of the experiment. Pentazocin administration significantly increased response time of the animal to reach 24 secs (after 0.5 hrs–1 hr). Its analgesic effect decreased with time but remained significant even after 6 hours (13.8 secs). All doses showed significant increase in the latency time of mice when compared to control group. As can be seen in Table 2, the maximum activities were obtained after 3 hours with 15.7, 16.8, 18, 18.8, 21.3, and 24 secs for 100, 200, and 400 mg/kg extract. The increase in latency time induced by the plant extract was maintained for 8 hours and remained significant after 24 hrs for 100, 200, and 400 mg/kg doses (Figure 2). Figure 3 demonstrated the relative activity of leaves extract with respect to the standard drug (pentazocine) in the hot plate method. All doses of leaves extract were more effective than pentazocine after 3.5 hrs. The maximum activity of leaves extract was achieved at 4 hours, where its relative activity to pentazocine reached 1.15, 1.1, 1.36, 1.4, and 1.62 for 50, 75, 100, 150, and 200 mg/kg, respectively. The analgesic effect of the extract was very close to pentazocine with relative activities greater than 1 for all doses after 3 hours. The peak in latency time response of pentazocine (after 30 min) was 14.33, while that of leaves extract was 14.12 secs (200 mg/kg) after 3 hours. The time of analgesic coverage calculated as AUC for all doses of extract (100, 200, and 400 mg/kg) was 54.2, 67.7, and 72.9 respectively, being greater than that of pentazocine 52.83, as can be seen in Table 3.

Table 2: Analgesic effect of different doses of *Andrographis paniculata* leaves extract by hot plate method in mice.

Treatment	Reaction time (sec, mean ± SEM)									
	0 hrs	0.5 hrs	1 hr	1.5 hrs	2 hrs	3 hrs	4 hrs	6 hrs	8 hrs	24 hrs
Control	9.4 ± 0.54	9.64 ± 0.91	9.82 ± 0.68	10.04 ± 0.42	10 ± 0.7	9.5 ± 0.65	10.44 ± 0.93	10.66 ± 0.65	10.2 ± 0.28	9.15 ± 0.35
Leaves extract 100 mg/kg	9.82 ± 0.95	12.84* ± 1.79	14.76** ± 2.38	16.76** ± 2.09	18.4** ± 1.29	20.2** ± 2.31	18.08** ± 1.04	16.18** ± 1.87	14.7* ± 0.71	12.8* ± 0.57
Leaves extract 200 mg/kg	8.83 ± 1	12.27 ± 1.92	14.77** ± 1.44	17.2** ± 2.35	20.27** ± 2.77	21.3** ± 3.11	18.87** ± 1.86	16.7** ± 0.44	15.83* ± 0.35	13.33* ± 0.61

Leaves extract 400 mg/kg	9.88 ± 0.75	13.37** ± 2.46	15.53** ± 1.04	20.03** ± 3.59	22.85** ± 4.4	24** ± 4.41	21.78** ± 3.9	17.68** ± 1.01	15.9* ± 0.99	14.15* ± 1.06
Pentazocine (5 mg/kg)	9.72 ± 0.68	24.05** ± 2.67	24.03** ± 2.4	22.08** ± 1.44	20.7** ± 2.69	18.15** ± 1.14	14* ± 2.39	13.8* ± 1.08	10.8 ± 0.28	9.4 ± 0.28

* P < 0.05; **P < 0.001 (n = 6).

Table 3: Comparison of AUC of different doses of *Andrographis paniculata* leaves extract and pentazocine.

Treatment	Tail flick method			Hot plate method		
	AUC	Response peak (sec)	Time (hrs)	AUC	Response peak (sec)	Time (hrs)
Pentazocine (5 mg/kg)	16.85	8.5	0.5	52.83	14.33	0.5
Leaves extract 100 mg/kg	23.38	4.59	3	54.26	10.38	3
Leaves extract 200 mg/kg	29.66	5.6	3	67.73	12.47	3
Leaves extract 400 mg/kg	33.58	7.18	3	72.96	14.12	3

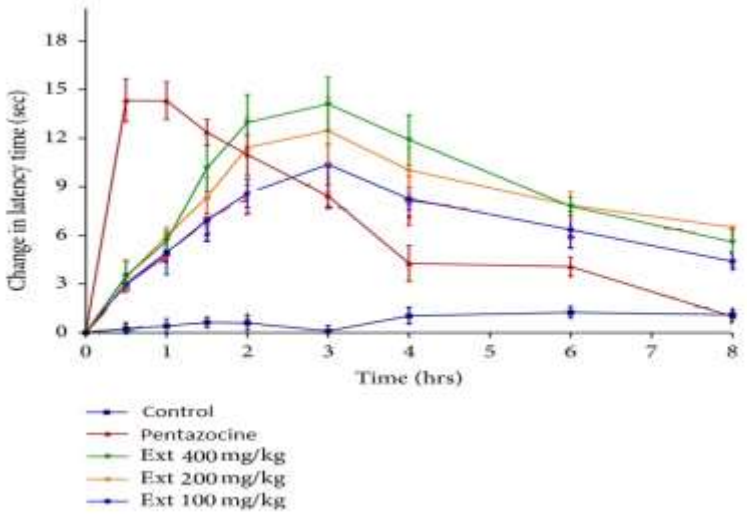


Figure 2: Effect of different doses of *Andrographis paniculata* leaves extract on change in latency time using hot plate test in mice.

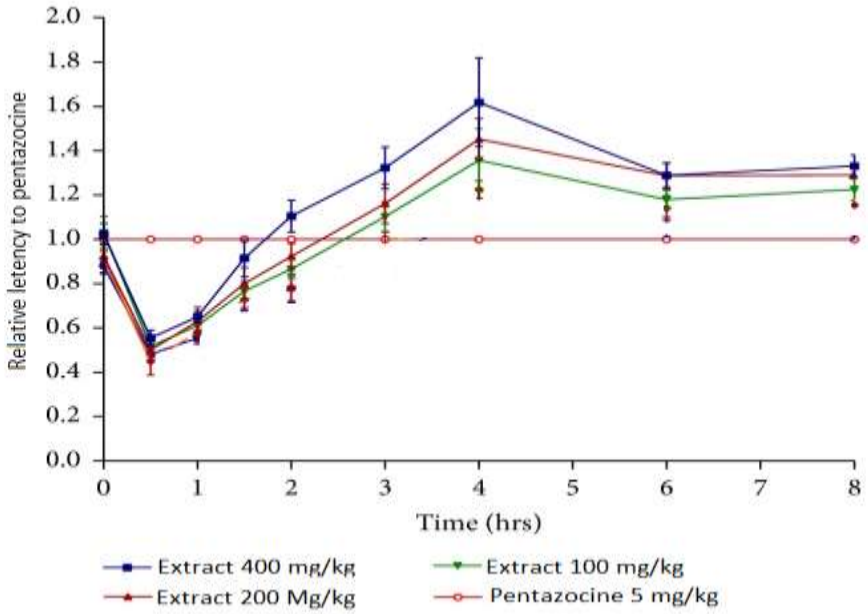


Figure 3: Relative activity of *Andrographis paniculata* leaves extract with respect to pentazocine in hot plate method.

(b) Effect on acetic acid-induced writhing responses

The analgesic effects of leaves extract were investigated against the writhing responses in mice induced with acetic acid. The average writhing number in the control group for 10 min was recorded as 100%. Leaves extract administration resulted in the reduction in the number of writhing compared to the control. Rats fed with 1000 mg/kg of leaves extract had the average writhing number of 67.22%, which was close to that of the acetylsalicylic acid group (58.09%). This result shows the analgesic effects of leaves extract against peripheral pain **(Figure 4).**

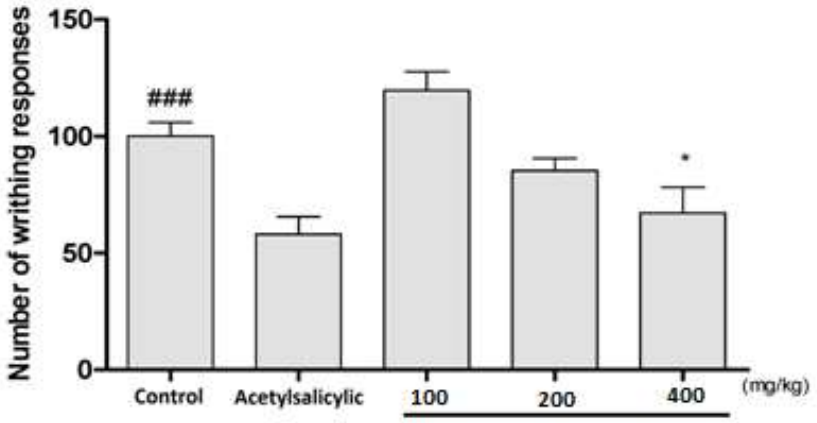


Figure 4: Effects of *Andrographis paniculata* leaves extract on writhing responses of acetic acid-induced mice. After 30 min of oral administration, every mouse was intraperitoneally injected with 0.7% acetic acid before 10 min counting. The number of mice was 7–8 per group; ### p < 0.001 vs. acetylsalicylic, * p < 0.05 vs. control by one-way ANOVA, Dunnett's test.

3.3. Anti-inflammatory activity

(A) Carrageenan-induced paw oedema in rats

The in-vivo anti-inflammatory activity was assessed by administering doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of the methanolic extract *Andrographis paniculata* leaves, in comparison with the standard diclofenac sodium at a dose of 10 mg/kg (Figure 5). The results indicated maximal activity at 14.63%, 25.64%, 33.33%, and 37.50% respectively, observed at a dose of 400 mg/kg across different time intervals. In comparison, diclofenac sodium exhibited activities of 16%, 27.77%, 41.93%, and 44.82% at a dose of 10 mg/kg (Table 4 and Figure 6). Notably, the methanolic extracts of *Andrographis paniculata* leaves demonstrated remarkable anti-inflammatory properties, particularly at a dose of 400mg/kg (Table 5 and Figure 7).

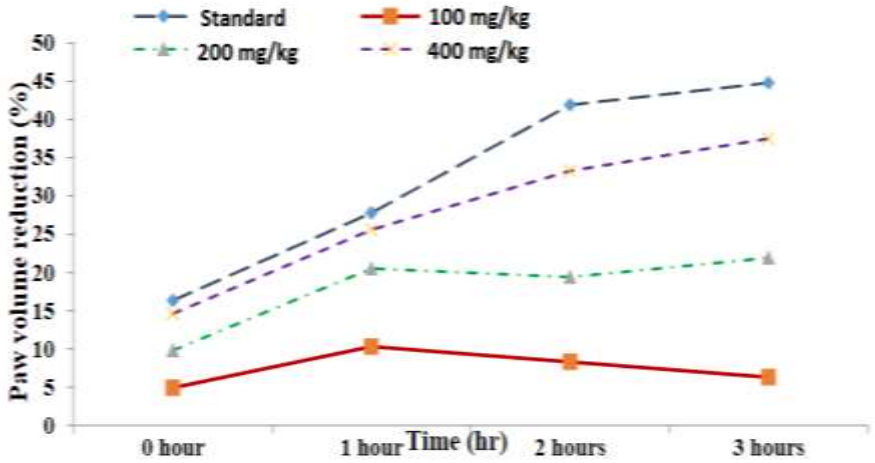


Figure 5: Paw volume reduction of *Andrographis paniculata* leaves extract at different concentration of doses was compared with the effect of Standard Diclofenac Sodium.

Table 4: Percentage inhibition of inflammation, induced by carrageenan.

Paw volume reduction (%				
Groups	**Time (h)**			
	0 hour	**1 hour**	**2 hour**	**3 hour**
Diclofenac	16%	27.77%	41.93%	44.82%

100 mg/kg	5%	10.25%	8.33%	6.25%
200 mg/kg	9.75%	20.51%	19.44%	21.87%
400 mg/kg	14.63%	25.64%	33.33%	37.50%

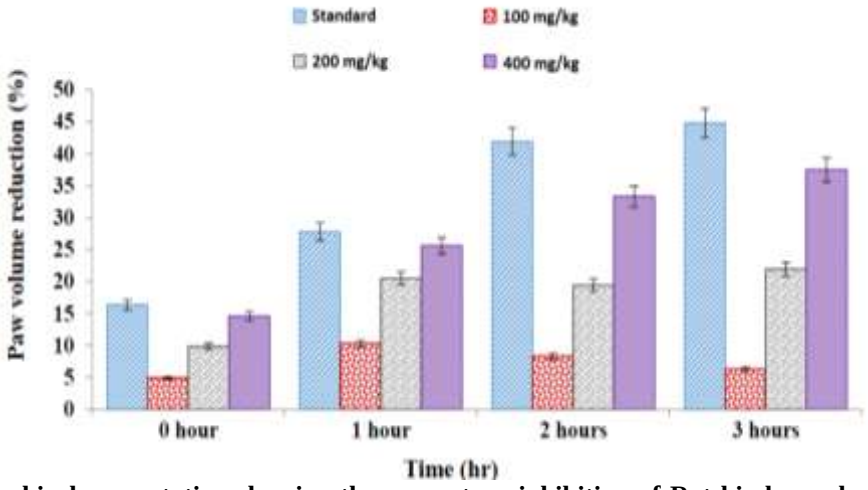


Figure 6: The graphical presentation showing the percentage inhibition of Rat hind paw healed from swelling due to Carrageenan injection.

Table 5: Anti-inflammatory activity by carrageenan induced paw edema (Mean ± SD) (n=5).

Carrageen induced edema (Volume in ml)				
Drug & extract (mg/kg)	**Time (h)**			
	0 hour	**1 hour**	**2 hour**	**3 hour**
Control	0.49±0.0260.	0.54±0.030	0.58±0.015	0.62±0.026
Diclofenac	0.41±0.020**	0.39±0.017**	0.36±0.017**	0.32±0.020**
100 mg/kg	0.39±0.017**	0.35±0.010**	0.33±0.020**	0.30±0.017**
200 mg/kg	0.37±0.026**	0.31±0.017**	0.29±0.026**	0.25±0.017**
400 mg/kg	0.35±0.017**	0.29±0.026**	0.24±0.017**	0.20±0.010**

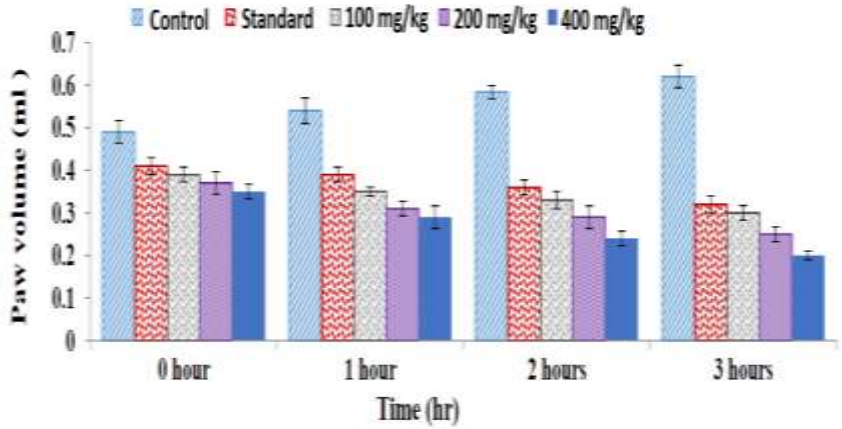


Figure 7: Graph showing results of Control, standard and methanolic extract of *Andrographis paniculata* the rat paw volume.

4. CONCLUSION

The present research work demonstrates that *Andrographis paniculata* leaves extract acts as a potent analgesic and anti-inflammatory agent. The analgesic activity of leaves extract may be due to its ability to activate opioid receptors in the central nervous system. It may also inhibit endogenous pain substances, which are involved in the peripheral analgesia. The analgesic activity of leaves extract may be due to the presence of Andrographolide, diterpenes 14-deoxyandrographolide, neoandrographolide, isoandrographolide, and 14-deoxy-11,12-didehydroandrographide. The anti-inflammatory property of methanolic extracts of *Andrographis paniculata* leaves**,** showed dose-dependent decrease in

paw edema volume in albino rats. These results substantiate the long-standing traditional medicinal applications of *Andrographis paniculata* and propose its viability as a natural substitute for synthetic analgesic and anti-inflammatory drug. Future investigations should focus on validating its effectiveness and safety in human subjects, as well as elucidating its underlying mechanisms.

5. REFERENCES

1. Ashok Kumar B., Laksham K., Jayaveera K., Vel M., Arun Kumar P., Vinod Kumar R., et al. "Pain management in mice using methanol extracts of three plants belongs to family *Amaranthaceae*" Asian Pacific Journal of Tropical Medicine, 2010; 527-530.
2. Otari K., Shete R., Upasani C., Adak V., Bagade M., Harpalani A. "Evaluation of anti-inflammatory and anti-arthritic activities of ethanolic extract of Vernonia Anthelmintica seeds" Journal of cell and tissue research, 2010; 10(2): 2269-2280.
3. Newman DJ Natural products as leads to potential drugs: an old process or the new hope for drug discovery? J Med Chem, 2008; 51: 2589–2599.
4. Beghyn T., Deprez-Poulain R., Willand N., Folleas B., Deprez B. "Natural compounds: leads or idea? Bioinspired molecules for drug discovery" Chemical Biology and Drug Design, 2008; 72: 3-15.
5. Low, M., Khoo, C.S., Münch, G. *et al.* An *in vitro* study of anti-inflammatory activity of standardised *Andrographis paniculata* extracts and pure andrographolide. *BMC Complement Altern Med*, 2015; **15**: 18.
6. Hilkatul Ilmi, Irfan Rayi Pamungkas, Lidya Tumewu, Achmad Fuad Hafid, Aty Widyawaruyanti. Analgesic and Antipyretic Activities of Ethyl Acetate Fraction Tablet of *Andrographis paniculata* in Animal Models. Evid Based Complement Alternat Med, 2021; 2021: 8848797.
7. Suebsasana S., Pongnaratorn P., Sattayasai J., Arkaravichien T., Tiamkao S., Aromdee C. Analgesic, antipyretic, anti-inflammatory and toxic effects of andrographolide derivatives in experimental animals. *Archives of Pharmacal Research*, 2009; 32(9): 1191–1200.
8. Giglio C. A., Defino H. L., da-Silva C. A., de-Souza A. S., Del Bel E. A. Behavioral and physiological methods for early quantitative assessment of spinal cord injury and prognosis in rats. Braz. J. Med. Biol. Res, 2006; 39: 1613–1623.
9. Turner, R and P Ebborn. *Analgesics: Screening Methods in Pharmacology*. New York: Academic Press, 1965; 100.
10. Turner R. A. Screening Methods in Pharmacology: Analgesic Agent. London, UK: Academic Press, 1965; 13.
11. Collier, H. O. J., Warner, B. T. & Skerry, R. Multiple toe-pinch method for testing analgesic drugs. British Journal of Pharmacology, 1961; 17: 28-40.
12. Hijazi M., El-Mallah A., Aboul-Ela M., Ellakany A. Evaluation of analgesic activity of papaver libanoticum extract in mice: involvement of opioids receptors. Evidence-Based Complementary and Alternative Medicine, 2017; 2017: 13.
13. Augustine Tandoh, Cynthia Amaning Danquah, Charles Kwaku Benneh, Donatus Wewura Adongo, Eric Boakye-Gyasi, Eric Woode. Effect of Diclofenac and Andrographolide Combination on Carrageenan-Induced Paw Edema and Hyperalgesia in Rats. Dose Response, 2022; 20(2): 15593258221103846.
14. Sisignano M, Parnham MJ, Geisslinger G. Drug repurposing for the development of novel analgesics. Trends in pharmacological sciences, 2016; 37(3): 172-183.