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Research Article

PHYTOCHEMICAL SCREENING AND ANTI-ARTHRITIC ACTIVITY OF METHANOLIC LEAVES EXTRACT OF *ANDROGRAPHIS PANICULATA*

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Abstract:
Andrographis paniculata leaves are a herb widely distributed in tropical areas of Asia and Australia. This herb is cultivated across the globe firmly as a vegetable source. *Andrographis paniculata* are used intraditional medicinal system as anti-inflammatory agent, liver tonic, cardiotonic, skin infection, antiulcer etc. Number of phytochemical and pharmacological work has been carried out on deferent partsof *Andrographis paniculata*, prime objective of the study to find out Anti-Arthritic activity of methanolic extract of *Andrographis paniculata*. Methanolic extracts are rich of Alkaloids, glycosides, Diterpenes, saponins, flavonoids and phenol are also responsible for inflammatory induced Antiarthritic activity, presence of secondary metabolite phyto-constituents encourages the current work. Results of the current study shows that *Andrographis paniculata* having significant anti-arthritic activity.

Keywords: Anti-arthritic, Carrageenan, Freund's adjuvant, Methanolic extract, *Andrographis paniculata.*

Introduction:

1. ARTHRITIS

Arthritis is the swelling and tenderness of one or more of your joints. The main symptoms of arthritis are joint pain and stiffness, which typically worsen with age. The most common types of arthritis are osteoarthritis and rheumatoid arthritis. Osteoarthritis causes cartilage — the hard, slippery tissue that covers the ends of bones where they form a joint — to break down. Rheumatoid arthritis is a disease in which the immune system attacks the joints, beginning with the lining of joints.Uric acid crystals, which form when there's too much uric acid in your blood, can cause gout. Infections or underlying disease, such as psoriasis or lupus, can cause other types of arthritis. Treatments vary depending on the type of arthritis. The main goals of arthritis treatments are to reduce symptoms and improve quality of life. There are over 100 types of arthritis (Reddi *et al.*, 2013). The most common forms are osteoarthritis (degeneccrative joint disease) andrheumatoid arthritis. Osteoarthritis usually occurs with age and affects the fingers, knees, and hips. Rheumatoid arthritis is anautoimmune disorder that often affects the hands and feet. Other types include gout, lupus, fibromyalgia, and septic arthritis. They are all types of rheumatic disease.

1.2 CLASSIFICATION

There are several diseases where joint pain is primary, and is considered the main feature generally when a person has "arthritis" it means that they have one of these diseases, which include:

- Osteoarthritis (Mitsuyama *et al.*, 2007; Kean *et al.*, 2004)
- Rheumatoid arthritis (Aletaha *et al.*, 2010)
- Gout and pseudo-gout (Mitra, 2012)
- Septic arthritis
- Ankylosing spondylitis
- Juvenile idiopathic arthritis
- Still's disease
- Psoriasis (Psoriatic arthritis) (Amherdt-Hoekstra *et al.*, 2010; Mease *et al.*, 2004).
- Reactive arthritis
- Ehlers-Danlos Syndrome
- Haemochromatosis
- Lyme disease

- Sjogren's disease
- Hashimoto's Thyroiditis
- Celiac disease
- Non-celiac gluten sensitivity
- Inflammatory bowel disease (including Crohn's disease and ulcerative colitis)
- Henoch–Schönlein purpura.
- Granulomatosis with polyangiitis (and many other vasculitis syndromes).
- Familial Mediterranean fever.
- Systemic lupus erythematosus.

An undifferentiated arthritis is an arthritis that does not fit into well-known clinical disease categories, possibly being an early stage of a definite rheumatic disease.

1.3 SIGNS AND SYMPTOMS

Pain, which can vary in severity, is a common symptom in virtually all types of arthritis. Other symptoms include swelling, joint stiffness and aching around the joint(s). Arthritic disorders like lupus and rheumatoid arthritis can affect other organs in the body, leading to a variety of symptoms. Symptoms may include:

- Inability to use the hand or walk
- Stiffness, which may be worse in the morning, or after use
- Malaise and fatigue
- Weight loss
- Poor sleep
- Muscle aches and pains
- Tenderness

Difficulty moving the joint

It is common in advanced arthritis for significant secondary changes to occur. For example, arthritic symptoms might make it difficult for a person to move around and/or exercise, which can lead to secondary effects, such as:

Muscle weakness.

Loss of flexibility.

Decreased aerobic fitness

These changes, in addition to the primary symptoms, can have a huge impact on quality of life.

Joint pain, stiffness, and swelling are the most common symptoms of arthritis. Your range of motion may also decrease, and you may experience redness of the skin around the joint. Many people with arthritis notice their symptoms are worst in the morning.

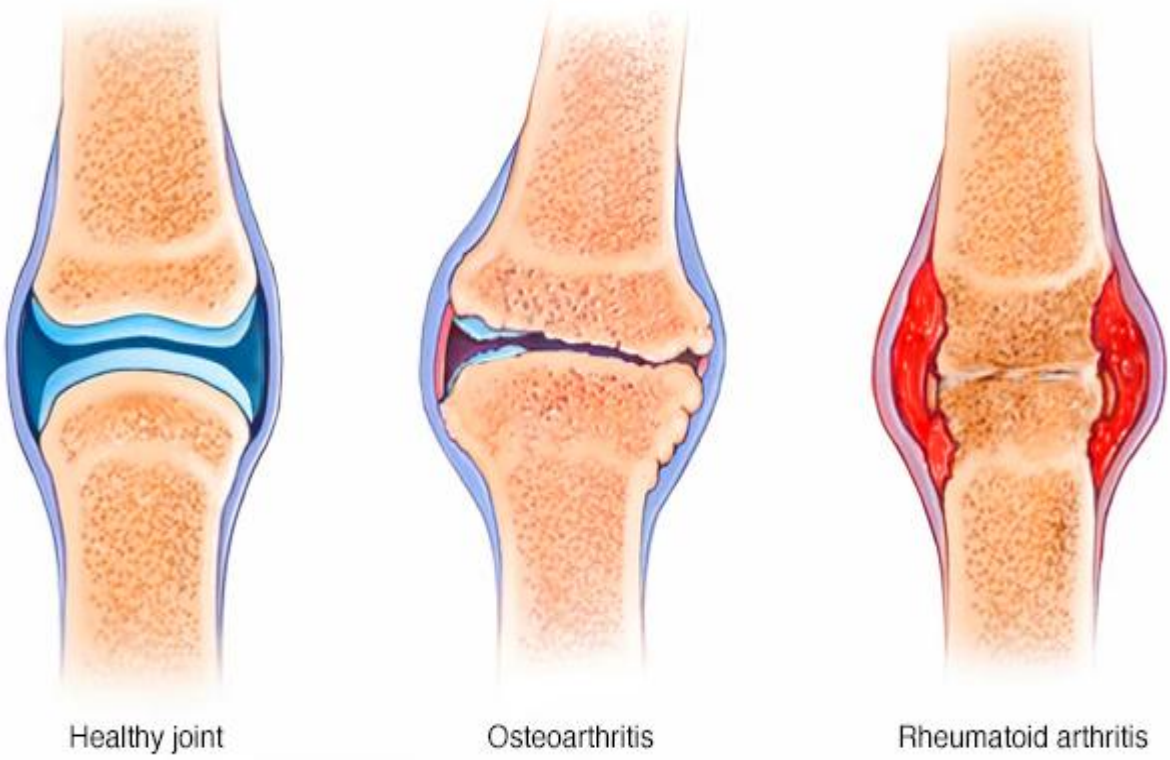

Figure 1: Healthy Joint and Arthriritis

1.4 CAUSES OF ARTHRITIS

Cartilage is a firm but flexible connective tissue in your joints. It protects the joints by absorbing the pressure and shock created when you move and put stress on them. A reduction in the normal amount of this cartilage tissue cause some forms of arthritis. Normal wear and tear causes OA, one of the most common forms of arthritis. An infection or injury to the joints can exacerbate this natural breakdown of cartilage tissue. Your risk of developing OA may be higher if you have a family history of the disease. Another common form of arthritis, RA, is an autoimmune disorder. It occurs when your body's immune system attacks the tissues of the body. These attacks affect the synovium, a soft tissue in your joints that produces a fluid that nourishes the cartilage and lubricates the joints. RA is a disease of the synovium that will invade and destroy a joint. It can eventually lead to the destruction of both bone and cartilage inside the joint.

- Injury - leading to degenerative arthritis.
- Abnormal metabolism - leading to gout and pseudogout.
- Inheritance - such as in osteoarthritis.
- Infections - such as in the arthritis of lyme disease
- Immune system dysfunction - such as in RA and SLE (Mansour, 2012).

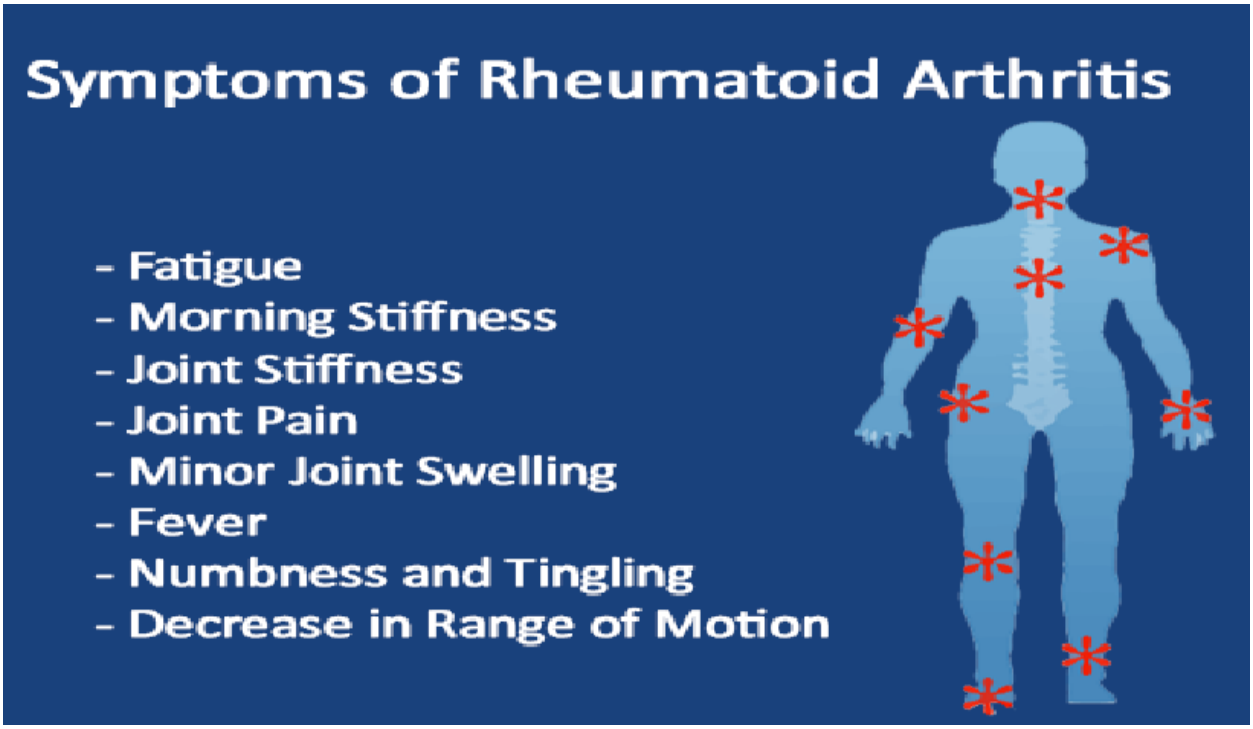

Figure 2: Symptoms of Arthritis

2. MATERIAL AND METHOD

2.1 Collection of plant material

Leaves of *Andrographis paniculata* were collected from rural area of Bhopal (M.P), India in the months of September, 2020.

2.2 Preparation of plant material for study

Plant materials (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then these plants materials were shade dried without any contamination for about 3 to 4 weeks. Dried plant materials were grinded using electronic grinder. Dried plant material was packed in air tight container till any further use.

2.3 Extraction process

50 gm dried powdered leaves of *Andrographis paniculata* has been extracted with methanolic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C. (Mukherjee, 2007).

2.4 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.5 Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in extracts (Khandelwal, 2005; Kokate, 1994). The *Andrographis paniculata* leaves extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of Alkaloids, glycosides, Diterpenes, saponins, flavonoids and phenol.

2.6 Total Phenolic content estimation

Procedure: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.7 Total flavonoids content estimation

Procedure: Determination of total flavonoids content was based on aluminium chloride method (Olufunmiso *et al.,* 2011). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.8 *In-Vivo* Anti-arthritis activity

2.8.1 Animals:-

Albino Wistar rats of either sex (150–200 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Animas were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 8.00 to 15.00 h. Separate group (n=6) of rat was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.8.2 Chemicals:-

Freund's complete adjuvant (Sigma-Aldrich Chemical Co.) was used for experiments.

2.8.3 Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2002). Methanolic extract of Leaves of *Andrographis paniculata* (250, 500, 1000 and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-arthritic effect.

2.8.4 Anti-arthritis activity

Freund's adjuvant induced arthritis in rats: Animals were divided into five groups containing six animals each. Arthritic syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw (Jaijesh *et al.,* 2009).

Group I served as normal and received 2% gum acacia

Group II served as arthritis control-untreated received 2% gum acacia,

Group III received Diclofenac sodium (15 mg/kg p.o) served as reference standard

Group IV received extract of methanolic extract of Leaves of *Andrographis paniculata* of doses of 200 mg/kg p.o.

Group V received extract of methanolic extract of Leaves of *Andrographis paniculata* of doses of 400 mg/kg p.o.

3. RESULT AND DISCUSSION

3.1 Determination of Percentage Yield

Table 1: % Yield of methanolic extract of *Andrographis paniculata*

S. No.	Part	% Yield (W/W)
1.	Leaves	2.9%

3.2 Result of Phytochemical screening of extract

Table 2: Phytochemical screening of methanolic extract of *Andrographis paniculata*

S. No.	Phytochemicals	Tests	Observation	Inference
1.	Alkaloids	Iodine Test	No Blue colour	+
		Wagner's Test	No reddish brown precipitate	+
		Dragendorff's Tests	No orange brown precipitate	+
2.	Flavonoids	NaOH Tests	Colourless	+
		Shinoda Tests	Colourless	+
3.	Glycosides	Keller-Kiliani Test	No Bluish green colour	+
4.	Phenols	Phenol Tests	No Blue colour	+
5.	Saponins	Foam Test	Layer of foam	+
6.	Tannins	Gelatin Test	No white precipitate	+
7.	Carbohydrates	Molisch's test	No violet colour	-
		Fehling's test	Reddish orange precipitate	-
8.	Proteins	Millon's test	No white precipitate	-
9	Phytosterol	Salkowski test	golden yellow color	+

3.3 Results of Estimation of Total Phenolic Contentsand Total flavonoid content

Table 3: Estimation of Total flavonoid content and Total Phenolic content

S. No.	Solvents→ Bioactive compound↓	Methanolic extract
1.	Total flavonoid (Quercetin equivalent (QE) mg/g)	0.94 ± 3.25
2.	Total Phenol (Gallic acid equivalent (mg/g)	12.79±5.38

3.4 Results Anti-arthritis activity

Table 4: Anti-arthritis activity of methanolic extract of Leaves of *Andrographis paniculata* against Freund's adjuvant induced arthritis in rats

Group	Dose of extract (mg/kg, p.o.)	Change in paw thickness (mm)±SD (% inhibition)			
		1 Day	**7 Day**	**14 Day**	**21 Day**
Group I (Normal control)	1% CMC (1 ml/kg body weight)	4.05 ± 0.16*	4.05 ± 0.16*	4.05 ± 0.09*	4.05 ± 0.08*
Group II (Negative control)	1% CMC (1 ml/kg body weight) +FCA (0.1 ml)	5.71 ± 0.04	13.64 ± 0.03	18.20 ± 0.02	23.01 ± 0.04
Group III (Standard control)	Diclofenac sodium (15 mg/kg) +FCA (0.1 ml)	4.49 ± 0.03* (21.23)	7.96 ± 0.03* (41.64)	7.50 ± 0.03* (58.78)	6.75 ± 0.04* (70.64)
Group IV (Treatment Control)	*Andrographis paniculata* leaves (200 mg/kg)) +FCA (0.1 ml)	4.73 ± 0.09* (12.62)	9.51 ± 0.04* (27.75)	11.40 ± 0.07* (33.24)	11.93 ± 0.04* (43.13)
Group V (Treatment Control)	*Andrographis paniculata* leaves (400 mg/kg) +FCA (0.1 ml)	4.25 ± 0.04* (19.23)	7.91± 0.03* (38.74)	7.24 ± 0.09* (57.89)	6.78 ± 0.05* (68. 34)

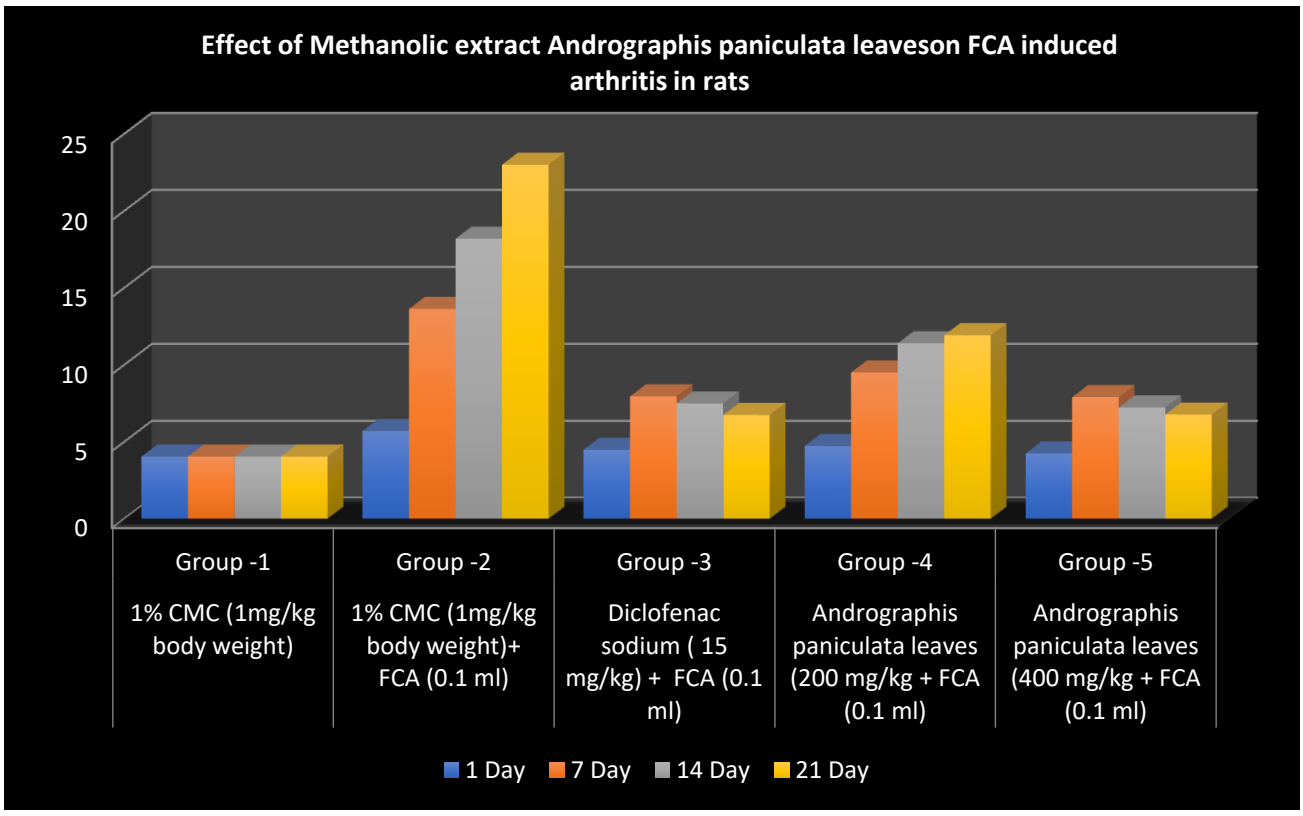

Figure 3: Anti-arthritis activity of methanolic extract of Leaves of *Andrographis paniculata* against Freund's adjuvant induced arthritis in rats.

In the current study, complete Freund's adjuvant induced arthritis in rats were selected to induce arthritis model, because it is the best and most widely employed empirical model for arthritis with clinical and laboratory features such as chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction and it has close similarities to human rheumatoid diseases (Singh and Majumdar, 1996). Oxygen derived free radicals and their products are known to play an important role in the pathogenesis of chronic inflammatory disorders. The importance of oxygen free radicals and related activated oxygen free intermediates in the pathogenesis of Rheumatoid arthritis has been identified with increasing incidence (Devi *et al.,* 2007). Paw swelling is one of the primary factors in evaluating the degree of inflammation and therapeutic efficacy of the drugs (Devi *et al.,* 2007). The initial inflammatory response will be produced within hours, but more vital clinical signs will be observed from the 7^{th} post-inoculation day and thereafter and the changes remain detectable for many weeks. The present study demonstrated that extract of methanolic extract of Leaves of *Andrographis paniculata* is able to suppress the swelling of the paws in both models i.e. arthritis. In the present study, rats was selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction. This may be due to the suppression of the inflammatory mediator released due to the induction of Complete Freund's Adjuvant (Tripathy *et al.,* 2009). From the results obtained, it can be said that herbal methanolic extract of Leaves of *Andrographis paniculata* possess significant antiarthritic property.

CONCLUSION

The whole plant or its specific parts (bark, leaf, seed and root) are known to have medicinal properties and have a long history of use by indigenous communities in India. The extracts were subjected to qualitative phytochemical screening using standard procedure. Phytochemical screening reveals the presences of alkaloids, glycosides, Diterpenes, saponins, flavonoids and phenol. Total phenolic and total flavonoid content was found 12.79±5.38 (mg/g) and 0.94 ± 3.25(mg/g) in *Andrographis paniculata* extract respectively.Complete Freund's Adjuvant induced arthritis and anti-arthritic property of the methonolic extract of Leaves of *Andrographis paniculata*. The effect of methonolic extract of Leaves of *Andrographis paniculata* were determined after administration at two dose level (200 and 400 mg/kg b.w.) in arthritis induced rats and assessed by histopathological studies.From the results, it may be concluded that herbal methonolic extract of Leaves of methonolic possess significant anti-arthritic effect may be due to the effect of antioxidants like Flavonoids, Phenols and Saponins present in the plant. It can be optimistic that the present work suggests an herbal drug of multiple therapeutic advantages and likely to be a powerful anti-arthritic drug.

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