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EVALUATION OF ANTI ARTHRITIC POTENTIAL OF HYDROALCOHOLIC LEAVES EXTRACT OF *WRIGHTIA TINCTORIA*

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ABSTRACT

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 medicinal value of this plant for the treatment of a large number of human ailments is mentioned in Ayurveda, Siddha, Unani and folk medicine In the last more than three decades, several studies have been carried out on this medicinal plant species to facilitate evidence in favor of its conventional uses. The extracts were subjected to qualitative phytochemical screening using standard procedure. Phytochemical screening reveals the presences of Flavonoids, Phenolics, Saponins. Total phenolic and total flavonoid content was found 1.077 and 0.586 in *Wrightia tinctoria* extract respectively. Complete Freund's Adjuvant induced arthritis and anti-arthritic property of the extract Hydroalcoholic extract of Leaves of *Wrightia tinctoria*. The effect of extract Hydroalcoholic extract of Leaves of *Wrightia tinctoria* were determined after administration at two dose level (100 and 200 mg/kg b.w.) in arthritis induced rats.

Key words: Phytochemical screening, *Wrightia tinctoria*, arthritis induced rats.

INTRODUCTION:

Arthritis is a term often used to mean any disorder that affects joints. Symptoms generally include joint pain and stiffness. Other symptoms may include redness, warmth, swelling, and decreased range of motion of the affected joints. In some types other organs are also affected. Onset can be gradual or sudden. There are over 100 types of arthritis (Reddi *et al.*, 2013). The most common forms are osteoarthritis (degeneccrative joint disease) and rheumatoid 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.

Treatment may include resting the joint and alternating between applying ice and heat. Weight loss and exercise may also be useful. Pain medication such as ibuprofen and paracetamol (acetaminophe) may be used. In some a joint replacement may be useful. Osteoarthritis affects more than 3.8% of people while rheumatoid arthritis affects about 0.24% of people (Woolf *et al.,* 2014). Gout affects about 1 to 2% of the Western population at some point in their lives (Bardin *et al.*, 2010). *Wrightia tinctoria* is an important medicinal plant used in the Indian system of medicine for the treatment of variety of diseases. *Wrightia tinctoria* R. Br. belongs to family Apocynaceae commonly called as Sweet Indrajao, Pala Indigo Plant, Dyer's Oleander. "Jaundice curative tree" in south India. Sweet Indrajao is a small, deciduous tree with a light gray, scaly smooth bark. In Siddha system of medicine, it is used for psoriasis and other skin diseases. Oil 777 prepared out of the fresh leaves of the plant has been assigned to analgesic, anti-inflammatory, and anti-pyretic activities and to be effective in the treatment of psoriasis. The plant is reported to contain presence of flavanoid, glycoflavones-iso-orientin, and phenolic acids. The various chemical constituents isolated from various parts of the plant are reported as 3,4-Seco-lup-20 (29)-en-3-oic acid, lupeol, stigmasterol and campetosterol, Indigotin, indirubin, tryptanthrin, isatin, anthranillate and rutin Triacontanol, Wrightial, cycloartenone, cycloeucalenol, β-amyrin, Alpha-Amyrin, and β-sitosterol, 14α-methylzymosterol. The present study evaluates the anti-arthritic activity of the leaves extract of *Wrightia tinctoria* in Freund's adjuvant induced arthritic Wistar albino rat model.

Extraction procedure

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs (Mukherjee, 2007).

Extraction by maceration process

55.8 gm dried powdered leaves of *Wrightia tinctoria* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Determination of percentage yield

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

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

Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in extracts (Khandelwal, 2005; Kokate, 1994).

Total Phenolic content estimation

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol

Preparation of Extract: 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.

Procedure: 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.

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method (Olufunmiso *et al.*, 2011).

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 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.

Procedure: 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.

***In-Vivo* Anti-arthritis activity**

Animals

Albino Wistar rats of either sex (150–200 g) 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 08.00 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.

Chemicals: -

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

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD) (OECD, 2002). Hydroalcoholic extract of Leaves of *Wrightia tinctoria* (5, 50, 300, 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.

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 Aspirin (200 mg/kg p.o) served as reference standard

Group IV received extract of Hydroalcoholic extract of Leaves of *Wrightia tinctoria* of doses of 100mg/kg p.o.

Group V received extract of Hydroalcoholic extract of Leaves of *Wrightia tinctoria* of doses of 200mg/kg p.o.

The drug treatment was started from 14th day of adjuvant induction and terminated on 28th day. The changes in paw volume was measured weekly by using Plethysmograph. At the end of experiment histopathology was done to check the inflammation.

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$, $P<0.01$, and $P<0.001$ were considered to be statistically significant.

RESULTS AND DISCUSSION

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. 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. The initial inflammatory response will be produced within hours, but more vital clinical signs will be observed from the 7th post-inoculation day and thereafter and the changes remain detectable for many weeks. The present study demonstrated that extract of Hydroalcoholic extract of Leaves of *Amaranthus spinosus* is able to suppress the swelling of the paws in both models i.e. arthritis. In the present study, rats were 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. From the results obtained, it can be said that herbal Hydroalcoholic extract of Leaves of *Wrightia tinctoria* possess significant antiarthritic property.

Table 1: % Yield of hydroalcoholic extract of *Wrightia tinctoria*

S. No.	Part	% Yield (W/W)
1.	Leaves	5.25

Table 2: Phytochemical screening of hydroalcoholic extract of *Wrightia tinctoria*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Hager's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenolics**	
	$Fecl_3$	+ve
5.	**Proteins and Amino acids**	
	Xanthoproteic test	-ve
6.	**Carbohydrates**	
	Fehling's test	-ve
7.	**Saponins**	
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve

Table 3: Total Phenolic and Total flavonoid content of *Wrightia tinctoria*

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	**Hydroalcoholic extract**	1.077	0.586

Table 4: Anti-arthritis activity of Hydroalcoholic extract of Leaves of *Wrightia tinctoria* against Freund's adjuvant induced arthritis in rats

Paw volume (mL)				
Group	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	0.25±0.60	0.25±0.50	0.25±0.50	0.25±0.40
Group II	0.65±0.23	0.85±0.55	0.90±0.25	0.93±0.35
Group III	0.60±0.15	0.58±0.15**	0.52±0.45***	0.35±0.56***
Group IV	0.70±0.15	0.68±0.12*	0.65±0.32*	0.20±0.21*
Group V	0.65±0.32**	0.62±0.21**	0.59±0.25***	0.55±0.45***

Values expressed as mean ± SEM (n=6) *P<0.05, **P<0.01, *** P<0.001 as compared to arthritis Control

CONCLUSION

From the results, it may be concluded that herbal Hydroalcoholic extract of Leaves of *Wrightia tinctoria* possess significant anti-arthritic effect may be due to the effect of antioxidants like Flavonoids, Poly Phenols and Saponins present in the plant. All these biological activities may be said to be a promising findings brought out by the present study. These contributions can be used as parameters for the authentication of plant as well as for developing newer drugs based on their activity. 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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