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PHYTOCHEMICAL SCREENING AND ANTI-ARTHRITIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF VERBASCUM THAPSUS

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ABSTRACT

Rheumatoid arthritis is a major auto immune disorder of body and affects the various joints which may lead to physical disability. The leaves of *Verbascum Thapsus* has long been used traditionally to treat rheumatoid arthritis. Though, it has not been pharmacologically assessed for rheumatoid arthritis. The current study phytochemical analysis of hydroalcoholic extract of *Verbascum Thapsus* leaves. Anti-arthritic potential was evaluated through Complete Freund's Adjuvant induced arthritis rats model at 250 mg/kg/p.o doses of the Hydro-ethanolic extracts of *Verbascum Thapsus* leaves using Diclofenac sodium as a standard drug. Apart from this we have evaluated paw edema parameters. The results revealed various arthritic protective mechanisms. Similarly plant exhibited dose dependent anti-arthritic effect with maximum activity observed at 500 mg/kg/ p.o. The results of Complete Freund's Adjuvant (CFA) model depicted better protection against paw edema and body weight alterations. Also *Verbascum Thapsus* leaves extracts remarkably altered locomotion of rats, and positive results were seen in hematological parameters and radiography. Moreover phytochemical analysis revealed phenols and flavanoids. By deeply analyzing the obtained results and data our research work supports the traditional use of *Verbascum Thapsus* leaves as a potential anti arthritic agent that may be proposed for rheumatoid arthritis treatment.

KEYWORDS: Rheumatoid arthritis, Complete freund's adjuvant, *Verbascum Thapsus*, Diclofenac sodium, Flavanoids.

1. INTRODUCTION

1.1 Rheumatoid arthritis

Rheumatoid arthritis is a chronic, systemic inflammatory disorder or a long term auto immune multisystem illness in which the body‟s immune system attacks the body‟s tissues and joints mistakenly causing an inflammatory synovitis which often progresses the destruction of joint ankylosis and articlular cartilage. An autoimmune disease is a condition which arises from an abnormal response to our normal immune system. The immune system is a host defence mechanism comprising complex organisation of cells and antibodies designed normally to “seek and destroy” invaders of the body. The synovium(inside of joints) is a thin delicate lining serves as an important source of nutrients for cartilage which thickens during RA resulting in inflammation and pain in and around the joints. Additionally, synovial cells synthesize joint lubricants and helps them move smoothly such as collagens, as well as fibronectin and hyaluronic acid that constitute the structural framework of the synovial interstitium.

Rheumatoid arthritis is influenced by the following factors such as gender, age, environmental factors and reproductive status, various studies demonstrate that genetic factors also play a major role on an individual‟s susceptibility to RA. It is characterized by periods of disease flares and remissions. Chronic inflammation of rheumatoid arthritis can cause permanent joint destruction and deformity. It leads to warm, swollen, painful and stiff joints which gets worsened following rest. Usually multiple joints of the fingers and hands, wrists, feet and knees typically gets affected in a symmetrical distribution (affecting both sides of the body). It mayalso affect other parts of the body and this may result in a low red blood cell count, inflammationaround the lungs, and inflammation around the heart.

1.2 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.

1.3 Causes of arthritis

- 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).

Most types of arthritis are caused by a combination of many factors working together, although some arthritis conditions have no obvious cause and appear to be unpredictable in their emergence.

2. EXPERIMENTAL WORK

2.1 Collection of plant material

Leaves of *Verbascum thapsus* were collected from Vindhya herbal nursery Bhopal (M.P), in the months of October, 2021.

2.2 Extraction procedure

55.8 gm dried powdered leaves of *Verbascum thapsus* has been extracted with hydro-ethanolic solvent (Ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

2.3 Determination of percentage yield

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

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

2.4 Phytochemical screening

The *Verbascum thapsus* 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 Carbohydrate, alkaloids, glycosides, phenols, flavonoids, Terpenoids, Saponins, Tannin.

2.5 Total phenolic content estimation

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

2.6 Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method.

2.7 *In-Vivo* Anti-arthritis activity

2.7.1 Animals

Albino Wistar rats of either sex (180–220 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.

2.7.2 Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2002). Hydro-ethanolic extract of Leaves of *Verbascum thapsus* (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.

2.7.3 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** (Normal control group): Rats received 1% CMC (1 ml/kg body weight) only daily for 14 days
- **Group II** (Negative control group): Received 1% CMC (1 ml/kg body weight) +FCA (0.1 ml) daily for 14 days
- **Group III** (Standard group): Diclofenac sodium 15 mg/kg suspended in CMC+FCA (0.1 ml) daily for 14 days
- **Group IV** (Treatment control group): Hydro-ethanolic extract of Leaves of *Verbascum thapsus* at dose of 250 mg/kg orally suspended in CMC +FCA (0.1 ml) daily for 14 days
- **Group V** (Treatment control group): Hydro-ethanolic extract of Leaves of *Verbascum thapsus* at dose of 500 mg/kg orally suspended in CMC +FCA (0.1 ml) daily for 14 day

The drug treatment was started from 14th day of adjuvant induction and terminated on 21st day. The changes in paw volume was measured weekly by using Plethysmograph. At the end of experiment histopathology was done to check the inflammation.

3. RESULTS AND DISCUSSION

3.1 Determination of percentage yield

Table 3.1: % Yield of hydro-ethanolic extract of *Verbascum Thapsus.*

S. No.	Part	% Yield (W/W)
1.	Leaves	15.79

3.2 Result of Phytochemical screening of extract

Table 3.2: Phytochemical screening of hydro-ethanolic extract of *Verbascum Thapsus.*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 +ve +ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve -ve
4.	**Phenolics** $Fecl_3$	 +ve

5.	**Carbohydrates** Fehling's test	-ve
	Glycoside Legals test	+ve
6.	**Saponins** Foam test	+ve
7.	**Diterpenes** Copper acetate test	-ve
8.	**Tannin** Gelatin Test	+ve

3.3 Results of estimation of total phenolic contents

Table 3.3: Total Phenolic and Total flavonoid content of *verbascum Thapsus.*

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Hydro-ethanolic extract	1.163	0.817

3.4 Results Anti-arthritis activity

Table 3.4: Anti-arthritis activity of Hydro-ethanolic extract of Leaves of *Verbascum thapsus* 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)	4.07 ± 0 .16*	4.07 ± 0.16*	4.07 ± 0.09*	4.07 ± 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*	7.83 ± 0.03*	7.23 ± 0.03*	6.22 ± 0.04*
Group IV (Treatment Control)	Verbascum thapsus (250 mg/kg) +FCA (0.1 ml)	4.38 ± 0 .09*	9.17 ± 0.04*	10.91 ± 0.07*	11.23 ± 0.04*
Group V (Treatment Control)	Verbascum thapsus (500 mg/kg) +FCA (0.1 ml)	4.29 ± 0 .04*	7.69± 0.03*	7.72 ± 0.09*	6.85 ± 0.05*

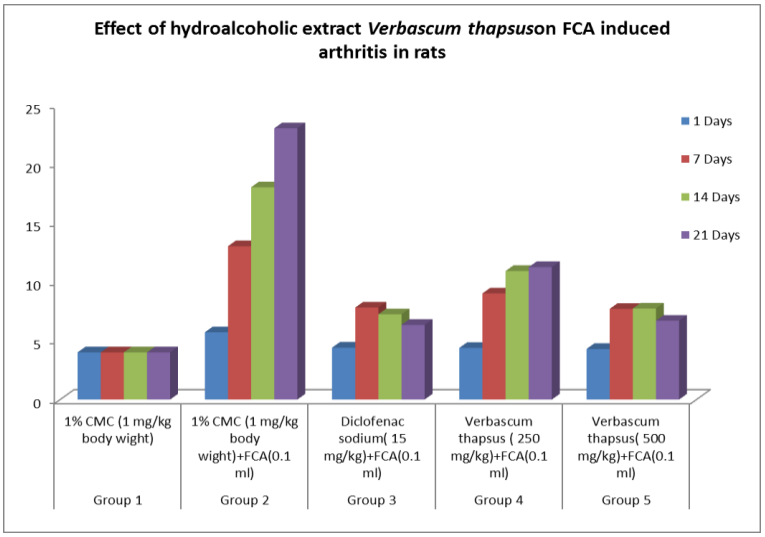

Figure 3.1: Anti-arthritis activity of Hydro-ethanolic extract of Leaves of *Verbascum thapsus* against Freund's adjuvant induced arthritis in rats.

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. 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. 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 7^{th} post-inoculation day and thereafter and the changes remain detectable for many weeks. The present study demonstrated that extract of Hydro-ethanolic extract of Leaves of *Verbascum thapsus* 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 Hydro-ethanolic extract of Leaves of *Verbascum thapsus* possess significant anti arthritic property.

CONCLUSION

Rheumatoid arthritis is a systemic autoimmune disease characterized by articular inflammation that eventually leads to the destruction of joints. Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1% of the population. Prevalence of RA increases with age, approaching 5% in women over the age of 55. The incidence and prevalence of RA is 2-3 times greater in women than in men. Effective treatment of RA has been impeded by a paucity of accurate diagnostic and prognostic tests, owing in part to the heterogeneity of the disease. The phytoconstituents present in it can to be cured arthritis effectively. From the results obtained in the present study, it may be concluded that leaves of *Verbascum Thapsus* possesses significant anti-arthritic activity. Further, research may be planned as an extension of this work which could prove leaves of *Verbascum Thapsus* as a potent anti-arthritic agent.

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