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Research Article

ANTI-ARTHRITIC ACTIVITY OF CHLOROFORM EXTRACT OF TUBERS OF *GLORIOSA SUPERBA* LINN

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ABSTRACT

Objective: Rheumatoid arthritis (RA) is a progressive, disabling and autoimmune disorder characterized by pain, swelling and stiffness of the synovial joints. *Gloriosa superba* Linn. (family-Liliaceae) is one of the herbaceous climbers distributed throughout Western Ghats and well documented traditionally in Ayurveda system of medicine for various ailments like inflammation, gout, gonorrhea, leprosy, rheumatoid arthritis, jaundice,abortifacient and helminthesis infections. The plant is highly valued in modern medicine owing to the presence of alkaloids, mainly colchicines. Despite its wide spread use in traditional medicine for treatment of gout and rheumatism, the present study was to evaluate the effect of chloroform extract of tubers of *Gloriosa superba* in Freund's complete Adjuvant (FCA) induced arthritis using albino rats.

Method: Arthritis was induced in male albino rats by intradermal injection of FCA (0.1 ml) in right hind paw. The groups were treated with test extract at a dose level of 30 mg/kg & 60 mg/kg/p.o, control (5% Tween- 80) and standard drug Diclofenac sodium(5.0 mg/kg/p.o.) from day 1-21. The changes in the paw edema and ankle joint diameter were measured by using plethysmometer and vernier caliper respectively on day 1,7,14, 21 and processed for histological assessment for evaluation of anti- arthritic activity. **Results:** The chloroform extract of tubers of *G superba* has shown a dose dependent and significantly decreased paw edema and ankle diameter in treated groups as compared with arthritic group.Acute treatment of rheumatoid rats with an extract of *G superba* tubers produced a significant inhibitory effect on rheumatoid arthritis (RA).

Conclusions: Our results contribute towards validation of the traditional use of *G superba* in the treatment of RA and other inflammatory joint disorders. The plant merits further investigation to prove the mechanism of action and to isolate its phytoconstituents responsible for the activity.

Keywords: Rheumatoid arthritis, Freund's complete Adjuvant,Colchicines, Synovial joint.

INTRODUCTION

Rheumatism is one of oldest known diseases of mankind and treatment-refractory chronic autoimmune disease. It affects approximately 5 million people worldwide of which 50% are to work beyond 10 years of diagnosis and is more common in women with female-to-male ratio being 3:1. No substantial progress has been made in achieving a permanent cure. Projections indicate that by 2020, almost 60 million people or about 20% of the population, will have arthritis[1].

Rheumatoid arthritis (RA) is a progressive, disabling, chronic multisystem disease that is characterized by pain, swelling and stiffness of the synovial joints. The exact etiology of this debilitating disease is not known, but it is believed to be the result of an autoimmune response of the body which can be triggered by a variety of external and genetic factors[2].

Among the many form of arthritis, rheumatism is a special one, where joints get targetedby immune system of self. This form of arthritis is a systemicinflammatory disease that attacks the synovial membranes surroundingthe lubricating fluid in the joint. The cartilage, along withthe bone structure is slowly

destroyed, leading to scar tissue formation and restriction the motion of joints[3].

Many medicines are clinically prescribed for treating this hard-to-cure illness.The medicines that are currently used for treatment of Rheumatoid arthritis are Disease modifying Anti-rheumatic drugs(DMARDs). Non-steroidal anti-inflammatory drugs (NSAIDs) and Steroids (Glucocorticoids).These are the group of medications commonly used in patients with rheumatoid arthritis. They work to decrease pain and inflammation, reduce or prevent joint damage, preserve the structure and function of the joints and improve the person's sense of well-being[4].

The side effects of these synthetic drugs are often deleterious, which includes gastrointestinal irritation, cardio vascular problem, drug dependency etc. However, these drugs is undesirable for prolonged treatments due to their poor efficacy, delayed onset of action, toxicity, high cost, long-term side effects and appearance of symptoms after discontinuation[5].

Therefore, much effort has been put into screening new therapeutic agents from natural products in the hope to reduce the risk of adverse events and slow down the progression of the disease. Many of indigenous plants have been claimed to be effective in the treatment of rheumatic disorders.*Gloriosa superba* Linn. (family-Liliaceae) is one of the endangered species among the medicinal plants which is a striking tuberous climber on bushes distributed throughout the tropical forests of Western Ghats in India [6].

The plant is well documented traditionally in Ayurveda system of medicine and is used in inflammations, gout, rheumatoid arthritis, gonorrhea and relieving fever[7].It is classified in Ayurvedic system as *Garbhapatani* (abotifacient) and used for promoting labour pains[8]. The medicinal importance of *G superba* is due to the presence of alkaloids in all parts of the plant mainly colchicines and its derivatives. The colchicine alkaloids are the drug of choice to relieve acute attack of gout[9].

By considering the presence of these active phyto-constituents in the plant extract, the present study was focused to document the anti-arthritic effect in experimental animal model.

MATERIAL AND METHODS

Collection and authentication of the plant material

The tubers of *G.superba* were collected from Balehonnur**,** Chikkamagalur district, Karnataka during flowering in rainy season. The plant material was taxonomically identified with the help of available literature and authenticated by taxonomist. The voucher specimen (KCD/4657) of the plant has been deposited in K.C.D. Herbarium, Department of Botany, Karnataka University, Dharwad, Karnataka.

Preparation of extract

Freshly collected tubers were shade dried at room temperature and coarsely powdered (# 22). The powdered plant material (500 g) was extracted with chloroform by continuous hot extraction method using Soxhlet apparatus for 18 h. The extract was concentrated in a rotary vacuum evaporator under reduced pressure and dried to obtain a dark brown semi-solid mass. The percentage yield of the extract was found to be 1.45 % w/w with respect to air dried plant material.

Phytochemical screening

Identification of the chemical constituents was carried out on the plant extract in order to determine the presence of various phyto-constituents using specific reagents[10].

Experimental animals

Anti-arthritic effect experiment was performed on-bread *Wister* albino rats (150-180 g)and Albino mice weighing 25-30 g were used for the acute toxicity study. The animals were fed with standard diet pellets and water *ad libitum.* They were housed in polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycles; temperature 25±2^{0}C and relative humidity 55±10%). Experiments were performed in accordance with the current guidelines of CPCSEA[11]norms after obtaining approval from the Institutional Animal Ethics Committee.

Acute toxicity (LD_{50}) studies

Acute toxicity study of the chloroform extractof tubers of *G.superba* (CEGS) was performed in overnight fasted albino mice by following fixed dose method as per OECD guidelines No.420 (anx-2d). Mortality & toxic symptoms in the treated animals were observed continuously for the first 3 h after dosing, periodically during the first 24 h and then daily observation for a total period of 14 days[12].

Evaluation of anti-arthritic activity[13]

The anti-arthritic activity the chloroform extractof tubers of *G.superba* (CEGS)was evaluated by using Freund´s complete adjuvant (FCA) induced arthritis**(**chronic inflammation model) in albino rats. An emulsion of 0.1 ml of FCA (inolive oil(1:2, v/v) at a concentration of 0.25mg heat killed *Mycobacterium tuberculosis* per ml of emulsion) was intra-dermal injected into

theplanter surface of rat's right hind foot. All the animals were injected withFreund´s complete adjuvant expect the normal control animals. The rats were randomly divided into five groups: normal control, arthritic control, standard and tests.

Group I - served as arthritic control

Group II - served as normal control (1% Tween- 80)

GroupIII - received standard drug Diclofenac sodium (5.0 mg/kg/p.o.)

Group IV- treated with CEGS (30 mg/kg body weight)

Group V - treated with CEGS (60 mg/kg body weight)

All the above treatments were given orally from day 0-28. The day of arthritis induction was considered as 0 day. The changes in the paw edema were measured by using Plethysmometer and Vernier calipers respectively, periodically on day 0,7,14 and 28.Arthritis severity was assessed from paw swelling and ankle swelling.The results were expressed as the percentage of hind paw swelling as compared with the initial hind paw thickness.

Statistical analysis

All results were expressed as mean±S.E.M. and analyzed for statisticalsignificance by one-way analysis of variance (ANOVA) followed by Dennett'st-test. *P values less than 0.05 were considered to be statistically significantas shown in Tables-1 and 2.

RESULTS

Phytoconstituents

Preliminary phytochemical analysis of the chloroform extract of *G superba* revealed the presence of alkaloids and phytosterols.

Acute toxicity studies

It was found that no mortality and changes in the behavior were observed up to dose 300 mg/kg body wt. Therefore, 1/5thand 1/10th of the maximal and sub-maximal tolerated safe CEGS doses (60 and 30 mg/kg body wt) were selected for screening of anti-arthritic activity.

Effect of CAGSon Freund's complete adjuvant-induced arthritisin rats

1. Measurement of paw swelling:

Subplantar injection of Freund's complete adjuvant (FCA) in the rat hind paw led to the development of arthritis which reached a peak edema on day 14 of the injection. The standard Diclofenac sodium (5.0 mg/kg/p.o.) was found to inhibit this edema toan extent of 65.17 % as shown in Table-1.The test extract, CEGS at the doses of 30 and 60 mg/kg body wt. p. o. has reduced swelling to the extent of 40.41 % and 56.86 %respectively as compared to control. This inhibition was dose dependent and found to be significant(*P<0.05).

2. Measurement of ankle joint diameter

The mean values of joint diameter using Vernier calipers are given in Table-2. The result indicates that CEGS at doses of 30 and 60 mg/kg body wtand standard Diclofenac sodium has shown 47.27 %, 52.27 % and 59.09 % inhibition respectively. These inhibition of joint diameter were significant (*P<0.05).

DISCUSSION

Rheumatoid arthritis (RA) is an incurable autoimmune disorder which leads towards disability and even premature death. Rheumatoid arthritis is a chronic inflammatory disease affecting about1%of the population in developed countries[14].

Gloriosa superba Linn. (Liliaceae) grows throughouttropicalregions in India and is a well known source of colchicines. Colchicine is aneffective drug treatment for intense pain associated with a gout attack[15].

The use of the adjuvant arthritis model offers an opportunity to study pathological changes in a variety of tissues other than the joints. Lysosomal acid hydrolases play an important role in inflammation associated with rheumatoid arthritis[16].

The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility, pause in body weight gain; hind paw and fore paw joint diameters increase. In the later, acute stages of disease (day 12+), rats with adjuvant arthritis are often relatively immobile due to the severity of pawswelling[17].

Our results showed that *G superba* extract significantly inhibited the development of chronic swelling induced by FCA. It seems that bacterial peptidoglycan and muramyl dipeptide are responsible for its induction.

Since the composition of bacterial adjuvant is complex and the immune response is a multi-stage process of intercellular co-operation, the mechanism is unclear. The model of adjuvant-induced arthritis in rats has been extensively used in the study of inflammatory processes and validated as a model of chronic pain[18].

The anti-rheumatic drugs do not stop the progression of this disease, but decrease the onset of disability by 30 %. Ibuprofen and Diclofenac sodium being the first line drugs offer little protection against tissue degeneration[19].

It is plausible that CEGS reduced inflammation by blocking both the lipo-oxygenase and cyclo-oxygenase pathways of arachidonic acid

metabolism. The observation that CEGS provided protection against adjuvant induced arthritis in rat development by significantly decreasing inflammation, increasing lysosomal stability and decreasing cartilage breakdown reduced inflammatory cytokines.

It was observed that *Gsuperba* possesses significant anti-arthritis action which provides a scientific basis to the claim of Ayurveda in the management of arthritis, inflammation, pain and similar ailments.

CONCLUSION

The present investigation provides scientific basis for the traditional use of *Gloriosa superba* Linn. as potential anti-arthritic plant. The plant merits further investigation to prove the mechanism of action and to isolate its phytoconstituents responsible for the activity.

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Table 1: Anti-arthritic activity of chloroform extract of tubers of *Gloriosa superba*(CEGS) on FCA-induced arthritis in rats by measuring paw swellingusing plethysmometer in different treatment groups

Group	Treatment	Dose mg/kg p.o.	Paw volume (ml)			Percentage Inhibition
			7th Day	14th Day	28th Day	
I	Arthritic Control	--	0.298 ±0.018	0.551 ±0.047	0.626 ±0.048	--
II	Normal Control	--	0.110 ±0.018	0.106 ± 0.014	0.108 ±0.012	--
III	Diclofenac sodium	5.0	0.236 ±0.022	0.470 ± 0.024	0.218 ±0.00*	65.17
IV	CEGS	30	0.285 ± 0.034	0.598 ± 0.062	0.373 ±0.062*	40.41
V	CEGS	60	0.306 ± 0.024	0.580 ± 0.0.27	0.270 ±0.57*	56.86

Data were analyzed by one-way ANOVA followed by Dunnet's t-test.n=6, values are expressed as mean ± SEM, *P<0.05 as compared to control.

Table 2: Anti-arthritic activity of chloroform extract of tubers of *Gloriosa superba*(CEGS) on FCA- induced arthritis in rats by measuring joint diameter (mm) using Vernier calipers

Group	Treatment	Dose mg/kg p.o.	Joint Diameter (mm)			Percentage Inhibition
			7th Day	14th Day	28th Day	
I	Arthritic Control	--	0.135 ±0.016	0.158 ± 0.017	0.220 ±0.015	--
II	Normal Control	--	0.110 ±0.033	0.020 ± 0.004	0.048 ±0.007	--
III	Diclofenac sodium	5.0	0.125 ±0.008	0.110 ± 0.014	0.090 ±0.03*	59.09
IV	CEGS	30	0.128 ± 0.013	0.122 ± 0.016	0.116 ±0.062*	47.27
V	CEGS	60	0.116 ± 0.014	0.110 ± 0.0.23	0.105 ±0.020*	52.27

Data were analyzed by one-way ANOVA followed by Dunnet's t-test; n=6, values are expressed as mean ± SEM, *P<0.05 as compared to control.

Fig. 1.1: Normal control

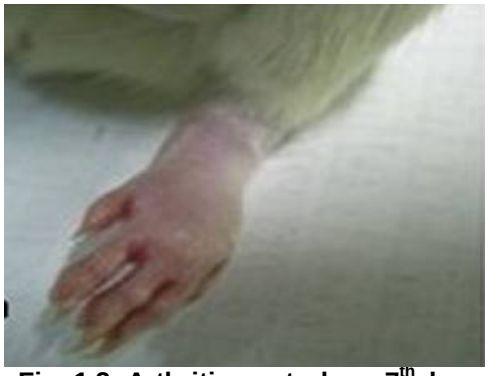

Fig. 1.2: Arthritic control on -7th day

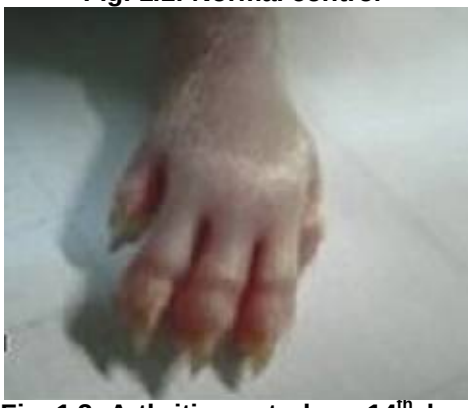

Fig. 1.3: Arthritic control -on 14th day

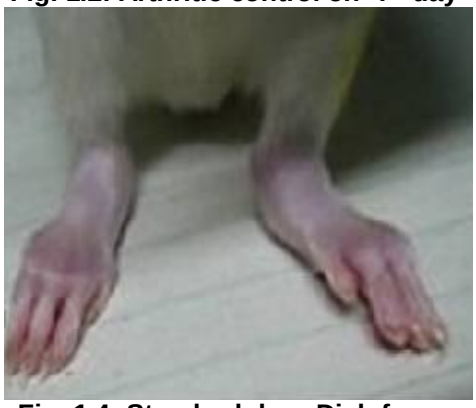

Fig. 1.4: Standard drug Diclofenac sodium(5.0 mg/kg)

Fig. 1.5: CEGS (30 mg/kg)

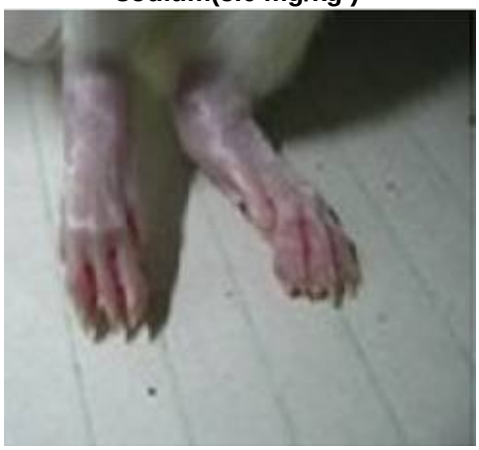

Fig. 1.6: CEGS (60mg/kg)

Fig. 1: Photograph of Anti-arthritic activity of chloroform extract of tubers of *Gloriosa superba* (CEGS)

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