Research Article

Ameliorative effect of *Alpinia galanga* rhizomes on Freund's complete adjuvant induced arthritis in mice

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Abstract

Arthritis is a inflammatory autoimmune disorder resulting in joint that is marked by swelling, pain, functional destruction and muscle wasting. The objective of present study was to evaluate rhizomes of *Alpinia galanga* for Freund's complete adjuvant induced arthritis in mice model. **Materials and methods**: Ethanolic extract with the dose of 100 and 200mg/kg was tested for antiarthritic activity using Freund's complete adjuvant model in mice. Activity was measured by paw edema measurement and observation of haematological parameters i.e. Leukocyte Count, Packed Cell Volume (PCV), and Rheumatoid Factor (RF), in the serum 28th days after CFA administration. **Results:** Antiarthritic potential of ethanolic extract found on dose dependent manner and 200mg/kg dose of extract was significantly observed. Results also supported by improved various inflammatory markers in plasma. **Conclusion:** Study can be concluded that the effect of ethanolic extract may be due to presence of flavonoids in the extract.

Keywords: *Alpinia galanga,* Arthritis, Freund's complete adjuvant

Introduction

Arthritis is a chronic autoimmune disease related to inflammation resulting in joint that is marked by swelling, pain, functional destruction and muscle wasting. It is illustrated by localized and systemic inflammation with eminent plasma concentrations of pro-inflammatory cytokines. Freund's complete adjuvant (CFA) is precise to enhance the humoral anti-type-II collage antibody response and to promote cellular immunity. However, it showed that, in mice, arthritis can be induced easily by a single injection of CFA. So, induction of this arthritis called as "adjuvant arthritis" (Morgan et al., 1997; Ratkay et al., 1993).

Alpinia galanga (Zingiberaceae) is commonly known as Kulanjan is distributed in throughout of India and Southeast Asia. It is well and richest source of essential oils like cineole, methyleugenol myrcene and methylcinnamate. It is also reported to presence of various flavonoids like galangin, alpinin, kampferide and 3-dioxy-4-methoxy flavones in rhizomes part (Cui, 2003). Traditionally it is used against rheumatism, bronchial catarrh, ulcers, throat infections, fever and dyspepsia. The rhizomes of the plant have been used in treatment of various ailments, such as stomach disorders, respiratory, cardiovascular and skin disorders (Chopra et al., 1956). Rhizomes have reported as antibacterial, antioxidant, immunostimulant, anti-protozoal, anti-fungal and expectorant action (Jain et al., 2012). In the present study we plan to evaluate ethanolic extract of *A. galanga* rhizomes for CFA induced arthritis in mice.

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Materials and methods

Plant materials and extraction

The rhizomes of *A. galanga* were collected from local market and authenticated in the department of Botany, Dr. H. S. Gour University, Sagar (M.P.) India. A herbarium of the plant was deposited for authentication in the department. The dried crude plant materials were powdered and subjected to extraction with different organic solvents. Defating of the plant materials was done with petroleum ether. Ethanol extract was obtained by hot extraction in soxhlet extractor. Ethanol extract was then subjected for qualitative chemical screening to detect the presence of phytoconstituents.

Animals

Mice of either sex (150-200 gm) were selected for *in vivo*

study. The food and water were supplied *ad libitum*. All the animals were kept under standard laboratory conditions in light and dark cycles and maintained under controlled temperature 27±20 for acclimatization. The experiment was conducted in accordance with Institutional Animal Ethical Committee (IAEC) CPCSEA, Government of India.

Acute toxicity study

Mice of either sex (150-200 gm) were used during investigation. The animals were fasted over night. According to the OECD guideline No-423 fixed dose method was adopted and the safest dose of the all extracts was 2000 mg/kg body weight calculated. On the basis of toxicity study, two different doses 100 and 200 mg/kg were selected for in vivo study.

Animals were divided in four groups each containing 6 animals per group. The treatment schedules of mice belonging to the different groups are shown below

·Group 1: Control (Complete Freund's adjuvant and normal saline solution)

·Group 2: Indomethacin against FCA induced arthritis (10mg/kg p.o)

·Group 3: Ethanol extract against FCA induced arthritis (100mg/kg p.o)

·Group 4: Ethanol extract against FCA induced arthritis (200mg/kg p.o)

Freund's complete adjuvant induced arthritis

The selected animals were anaesthetized and one ankle joint will be injected with 0.1 ml of Freund's complete adjuvant (FCA) containing 0.1mg *Mycobacterium tuberculosis*; in another the contra lateral knee was injected 0.1 ml (0.9%) saline. The animals were allowed to free for recover. Animals were examined for visual observations of arthritis in peripheral joints and scores for arthritis (Ratkay et al., 1993). A confirmation of arthritic condition was considered when significant changes in redness and swelling were noticed in the paw. The clinical severity of the arthritis in each paw was quantified weakly by quantification of the paw volume change upto day 28th. Animals paw volume was measured using mercury plethysmometer. Dosing with standard drug Indomethacin (10 mg/kg body weight) and ethanol extracts (100 and 200 mg/kg) was started on the same day and continued up to 28th day.

Hematological parameters

Blood aamples collected from different groups were tested for measurement of Leukocyte Count, Packed Cell Volume (PCV), and Rheumatoid Factor (RF), in the serum 28th days after CFA administration.

Statistical analysis

Pharmacological data were represented as the mean ±S.E.M. for six rats and data were evaluated using the Tukey test. Values of P <0.05 were considered to be statistically significant.

Results

Phytochemical study

In the study of phytochemical screening of ethanol extract from *A. galanga* rhizomes revealed the presence of flavonoids, alkaloids, glycosides, proteins and carbohydrates. On the basis of acute toxicity study two consequence doses 100 and 200 mg/kg were selected for *in vivo* study.

FCA induced arthritis

The present study contains an in vivo experiment for evaluation of anti arthritis activity of *A. galanga* rhizomes. The mice received a single injection of CFA on initial day and were examined daily up to 28th day for symptoms of arthritis. A reference group was used for comparison. Redness and swelling in to the joints initiated to appear on 2 days earlier in mice injected with CFA alone. After the time duration of the experiment, mice injected with CFA alone developed arthritis, mice treated with ethanol extract and reference drug showed minor swelling, reduced oedema and redness. The control group showed gradually reduced oedema with time up to 28th days, but very slowly. The ethanol extract showed oedema inhibition in dose dependent manner. Ethanol extract with 200mh/kg showed most effective for oedema inhibition in mice (Table 1).

Table 1. Effect of *A. galanga* ethanol extract on FCA induced mice paw edema

Treatment Groups	Paw Edema (ml) (Mean ±SEM)				
	0 week	1st week	2nd week	3rd week	4th week
Control with Saline solution	0.076±0.042	3.22±0.059	3.57±0.072	3.50±0.0428	3.07±0.068
Indomethacin against FCA induced arthritis (10mg/kg p.o)	0.062±0.072	1.43±0.073	1.14±0.027	0.85±0.037	0.27±0.061
Ethanol extract against FCA induced arthritis (100mg/kg p.o)	0.073±0.052	1.57±0.049	1.47±0.043	1.07±0.043	0.86±0.027
Ethanol extract against FCA induced arthritis (200mg/kg p.o)	0.048±0.061	1.23±0.072	1.14±0.072	0.84±0.018	0.46±0.061

Both doses of the ethanol extract 100 and 200mg/kg produced a significant increase in the RF and Leukocyte count compared with untreated control group. There was no significant difference in the WBC counts. RF was negative in both treated and untreated rats (Table 2).

Discussion and conclusion

The results of the paw volume and inflammatory markers

from the site of CFA administration to the other paw can be correlated by a migratory phenomenon in CFA induced arthritis.

The possible mechanism by which the ethanol extract exhibited anti-arthritic activity could be to suppress the activation of nuclear factor (NF-kB). In inflammatory diseases, NF-kB is prominent and is responsible for improved expression of several pro-inflammatory mediators (Morgan et al., 2005; Riehemann et al., 1999).

Table 2. Effects of ethanol extract of *A. galanga* on hematological parameters

Animal groups	Rheumatoid Factor (IU/ml)	Leukocyte Count (Cu.mm/ml)	Packed Cell Volume (%)
Control with Saline solution	14.26±1.25	3145±352.4	29.24±2.65
Indomethacin against FCA induced arthritis (10mg/kg p.o)	42.18±3.26	4523±402.3	42.17±4.25
Ethanol extract against FCA induced arthritis (100mg/kg p.o)	35.24±2.56	5624±428.7	45.32±3.95
Ethanol extract against FCA induced arthritis (200mg/kg p.o)	41.27±3.14	5378±395.2	46.20±4.12

In the present study, we select complete Freund's adjuvant induced arthritis method in mice for induction of arthritis, because it is most suited and widely in use model for arthritis with clinical and laboratory utility due to accumulation of inflammatory cells, corrosion of joint cartilage and bone destruction comparable to human rheumatoid diseases (Singh and Majumdar, 1996).

Oxygen derived free radicals are known to play an important role in the pathogenesis of different inflammatory disorders. The role of oxygen free radicals and associated activated oxygen free intermediates in the pathogenesis of arthritis has been identified with growing occurrence (Devi et al., 2007). Rhizomes of *A. galanga* already reported to have petent antioxidant activity (Jain et al., 2012). In conclusion, the anti arthritic effect of ethanol extract of *A. galanga* rhizomes showed in dose dependent manner. The plant contains various flavonoids constituents that may be responsible for the accelerated activity and mechanism behind may be its free radical scavenging action.

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Conflict of interest

Authors did not have any conflict of interest.

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