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Research Article

EVALUATION OF ANTIARTHRITIC ACTIVITY OF *SCHISANDRA GRANDIFLORA* AGAINST FORMALDEHYDE-INDUCED ARTHRITIS IN RATS

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ABSTRACT

Objective: *Schisandra grandiflora* is a plant, reported for its variety of ethnic medicinal uses. The current study was undertaken to evaluate the antiarthritic activity of *S. grandiflora* against formaldehyde-induced arthritis in rats.

Methods: The plant powder successively extracted with water and subjected for phytochemical screening to identify the different phytochemical constituents. LD50 study is conducted for the aqueous extract up to the dose level of 2000 mg/kg by following the method of OECD guidelines no. 425.

Results: Preliminary phytochemical studies revealed the presence of terpenoids, flavonoids, alkaloids, and glycosides in aqueous extract of *S. grandiflora.* No mortality was observed with the aqueous up to the maximum dose level of 2000 mg/kg. Further, aqueous extracts at 100 and 200 mg/kg, p.o but not with 100 mg/kg p.o dose significant ($p<0.01$) reduced paw edema in formaldehyde-induced arthritis in rats.

Conclusion: The present study revealed the antiarthritic activity extract of *S. grandiflora.* Moreover, the activity is showed the presence of phytochemical constituents such as terpenoids, alkaloids, glycosides, and flavonoids as these phytochemical constituents.

Keywords: *Schisandra grandiflora*, Formaldehyde, Indomethacin.

INTRODUCTION

Arthritis means arth=joint and itis=inflammation, a term used to describe a number of painful conditions of the joint bones. Rheumatoid arthritis (RA) is often associated with older people but can also affect children. About 1 in 1000 children develops arthritis, often called as juvenile idiopathic arthritis [1].

RA

It is an autoimmune chronic disease characterized by synovial inflammation of the joints. With proliferation of the synovium, progressive erosion of cartilage, and bone particularly affecting elderly people which lead to massive bone destruction with consequent inflammation and pain. Extra-articular immunologic abnormalities may extend to involve other organ systems as well [2]. At least 70% of patients with RA have positive rheumatoid factor (RF) autoantibodies directed against antigenic determinants on the fragment of immunoglobulin (Ig) G. RF is an autoantibody directed against the Fc region of human Ig G. Deposits of RF linked with Ig G occur in various tissues, such as the synovium or joints, interfere with the normal function of the joint to promote local inflammation, resulting in tissue damages, and sometimes damage to blood vessels in the affected area. The RF test is used as a diagnostic marker for RA since it is present and is associated with developing RA increased risk in people with mild arthritic symptoms. Higher levels are also detected in more severe forms of the disease, a condition that is a severe prognostic factor for patients. The normal range of RF is from 0 to 20 µ/ml. RF above 20 µ/ml is not considered enough to diagnose RA, as these other reasons the RF level may be elevated. Some conditions of medical procedures that can raise RF levels include other autoimmune diseases [3].

- Certain chronic infections
- Diabetes
- Cancer.

It is important to note that once the RF level is elevated, it will often remain so even if the disease goes into remission.

METHODS

Plant material and preparation of extract

Schisandra grandiflora: Belongs to the family Schisandraceae

Collection of plant

The fruits of *S. grandiflora* plant were collected from the Himalayas and authenticated by Central Research Institute of Unani Medicine, Hyderabad (Authentication no: SMPU/CRI-Hyd 13195).

Plant drying

The fruits of *S. grandiflora* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no. #30. Dried samples were extracted with ethanol using Soxhlet process. The extracts were concentrated under reduced pressure using rotavapor.

Method of extraction of plant material

Hot continuous extraction (Soxhlet)

The freshly fruits are collected from *S. grandiflora*. This fruit was chopped, shade dried, and roughly powered, it was taken and placed in thimble made up of filter paper and inserted into the broad central tube of extractor and ethanol is located in the round bottom flask and carry to its boiling point 56°C. Its vapor passed through the larger right-hand tube into the upper part of one extractor and then to the condenser where they condensed and dropped back on to the drug. During the period, the soluble constituents are extracted when the level of the extract reaches the top of the siphon tube. The entire volume of extract siphons over into the flask and the process was continued until the during is completely extracted then the extract processed for evaporation, after the evaporation, the semi-solid jelly is formed [4].

Phytochemical screening

Chemicals and apparatus

Formaldehyde was purchased from Finar Fine Chemicals, Hyderabad, India. Other chemicals and reagents used were procured from Central Drug House, New Delhi. Apparatus such as the metabolic cage, electronic balance (electronic compact scale Virgo), ultraviolet spectrophotometer (Shimadzu), microcentrifuge (Elektrotechnik Ltd.), and incubator (Teknik AN10) was used in this study. Various kits for biochemical estimation of urine and serum were purchased from M/S Excel Diagnostics Pvt. Ltd., Hyderabad.

Animals

Albino Wistar rats weighing between 100 and 150 g were procured from Sainath Agencies Laboratory Animals, Bapujinagar, Musheerabad, Hyderabad, for the experimental purpose; then, the animals were acclimatized for 7 days under standard husbandry conditions as:

Room temperature: 24±2°C, humidity: 40–50%, and light/dark cycle: 12:12 h.

Table 1: Phytochemical screening of *Schisandra grandiflora* extract

Constituent	Result
Alkaloid	+
Flavonoids	+
Terpenoids	+
Glycosides	+

Fig. 1: Extraction of *Schisandra grandiflora* powder by Soxhlet

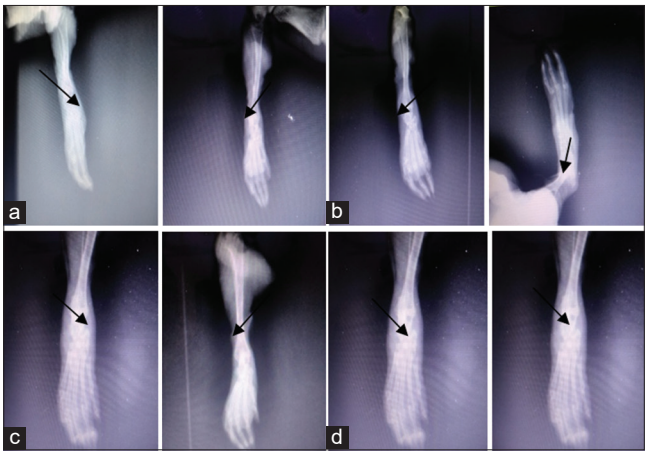

Fig. 2: (a) Test (low dose) (b) test (high dose) (c) standard (d) induced

Throughout the period of the experimental study, the animals were provided standard rodent pellet and distilled water. The study protocol was approved by the Institutional Animal Ethics Committee (Approval No: vcp/cology/001/11/2017) which was constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India [5,6].

Induction of arthritis

Formaldehyde-induced arthritis

Principle

After the injection of formaldehyde the growth of edema in the paw of the rat, the histamine discharge at the site of injection. At the site of administration, the formaldehyde-induced arthritis by denaturing protein produces immunological reaction against product degraded [7].

Experimental procedure

Albino rats weighing between 100 and 150 g were divided into four groups of four rats in each.

- The first group served as normal control, which was given with distilled water only
- The second group served as toxicant control, given with 0.1 ml of formaldehyde (2% v/v) into the hind paw
- The third group served as standard drug which was given with indomethacin (10 mg/kg)
- The fourth group served as test group treated with *S. grandiflora* plant extract at a dose of low (100 mg/kg) and high (200 mg/kg). Groups B, C, and D were intoxicated with 0.1 ml of formaldehyde (2% v/v). Daily the paw volume was measured for the next 10 days [8].

Paw volume

The rat paw diameter was measured using plethysmometer on days 1, 2, 4, 6, 8, and 10 of experimental period. On the 10^{th} day, animals were sacrificed from the retro-orbital puncture; the blood was collected. The biochemical parameter C-reactive protein (CRP) is estimated using diagnostic kit. Radiological examination was done for the knee joints on day 10 after the last dose administration of test extract and standard drug [9].

Acute toxicity test

The study for acute oral toxicity of test extract of *S. grandiflora* was determined in albino mice weighing between 18 and 22 g those maintained under standard husbandry conditions. Animals were fasted before dosing. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. Animals were administered with single dose of extract and observed for its mortality during the short-term period toxicity. Based on the extracts of short-term toxicity profile, the doses of the next animals were determined as per the OECD guidelines no. 425. All individual animals are observed at least once during the first 30 min after dosing, periodically during the first 24 h, and daily after that, for a total of 10 days [10].

Collection of blood samples

At the end of the study, the blood was collected from all animals through retro-orbital puncture into centrifuge tubes under the influence of mild anesthesia serum was separated by centrifugation at 3000 rpm for 10 min and was stored at room temperature −20°C for further biochemical assay.

Radiographic (X-ray) investigations

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the previous radiographic sign, whereas important radiographic changes such as bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis. The radiographic description of the rat joints in formaldehyde-induced arthritis model is shown in below figure. In formaldehyde-induced arthritic rat, soft tissue swelling along with the reduction of the joint spaces was observed which implies the bony destruction in arthritic condition. The standard drug indomethacin (10 mg/kg) treated groups have prevented this

Table 2:

Treatment	Paw volume in ml (mean±standard deviation)										
	0th	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Control	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01	0.92±0.01
Induced	0.92±0.01	1.85±0.05	1.85±0.05	1.86±0.10	1.86±0.10	1.82±0.10	1.82±0.10	1.82±0.10	1.80±0.12	1.78±0.13	1.77±0.15
Standard	0.92±0.01	1.71±0.07	1.71±0.07	1.83±0.07	1.71±0.05	1.68±0.02	1.64±0.08	1.44±0.09	1.19±0.02	1.19±0.02	1.16±0.04
Test (low)	0.92±0.01	1.8±0.06	1.8±0.06	1.88±0.04	1.83±0.02	1.69±0.03	1.70±0.03	1.62±0.02	1.62±0.02	1.54±0.03	1.53±0.04
Test (high)	0.92±0.01	1.74±0.03	1.74±0.03	1.74±0.03	1.66±0.03	1.65±0.02	1.62±0.02	1.52±0.02	1.43±0.01	1.36±0.02	1.23±0.04

n=4, significant at p<0.05*, p<0.01** AQESG: Aqueous extract of *Schisandra grandiflora*

Table 3:

Treatment	% change in edema volume (mean±standard deviation)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Induced	49.92±1.74	49.92±1.74	50.24±1.84	50.24±1.84	49.22±1.57	49.22±1.57	49.15±1.55	48.44±1.31	48.04±1.63	47.45±1.22
Standard	45.89±2.48	45.89±2.48	49.62±0.98	45.91±2.41	44.44±0.58	43.47±2.13	35.67±2.29	22.26±1.61	22.26±1.61	21.43±1.43
Test (low)	48.56±1.72	48.56±1.72	50.95±1.92	49.64±1.78	45.61±1.43	45.61±1.43	43.06±1.77	41.06±1.67	39.02±1.63	35.51±0.99
Test (high)	46.81±1.75	46.81±1.75	46.81±1.75	44.37±1.71	43.90±1.22	43.03±1.41	39.32±1.91	35.53±2.06	32.07±2.59	25.14±1.58

n=4, significant at p<0.05*, p<0.01** AQESG: Aqueous extract of *Schisandra grandiflora*

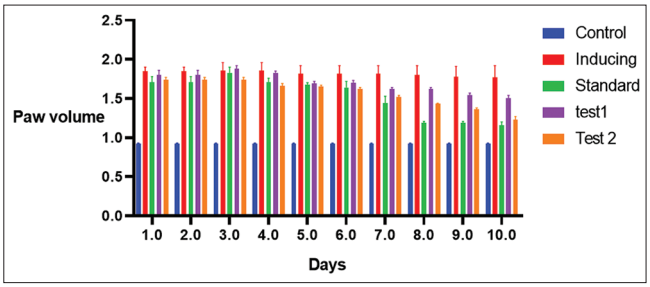

Fig. 3: Effect of formaldehyde-induced arthritis in rats percentage inhibition of paw edema in rats

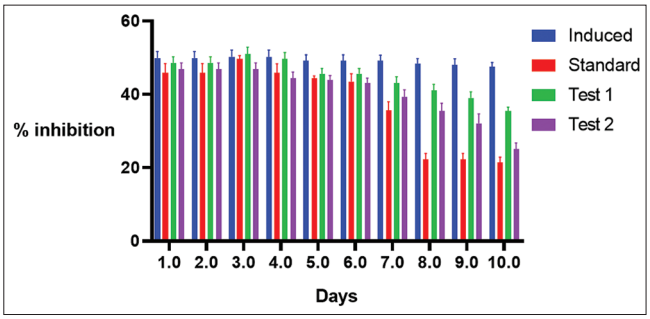

Fig. 4: Percentage inhibition of paw edema

bony destruction and also there is no swelling of the joint. The plant *S. grandiflora* treatment for 10 days has shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared with formaldehyde-induced group [11,12].

Statistical analysis

The values were expressed as mean ± standard deviation from four animals; the results were subjected to statistical analysis using one-way ANOVA followed by GraphPad Prism software. p<0.01 was considered as a statistically significant.

RESULTS

Formaldehyde-treated group is noted with a significant increase in paw volume from the 1st day to 10th day of the experimental study; on the 0th day, normal paw volume is recorded as 0.92 ml and after challenge with formaldehyde, the volume is gradually increased from the 1st day to 10th day with a minimum and maximum of 1.85 ml and 1.77 ml, respectively. The percentage increase in paw volume is noted as 49.92% and 47.45% on the 1st and 10th days of the study, respectively.

Indomethacin treatment (10 mg/kg) before formaldehyde challenge significantly contained raises the paw volume from the 1st day to 10th day of the experimental study. The percentage inhibition in paw volume is noted as 45.89% and 21.43%, respectively, on the 1st and 10th days of the study.

ALESG with two doses, i.e., 100 and 200 mg/kg when administered before formaldehyde has shown significant and dose-dependent percentage inhibition in paw volume from the 1st day to 10th day of the experimental study. The percentage inhibition of paw volume is recorded as minimum and maximum with the two different doses at the 1st and 10th days of the study are 48.56% and 39.51% and 46.81% and 25.14% of study, respectively.

Serum CRP

The administration of formaldehyde to rats resulted in elevation of serum CRP levels significantly than the 0th day. In treatment groups of 5th day and 10th day, significant decrease in serum CRP levels was observed. The significant dose-dependent reduction of serum CRP levels was also observed between low-dose and high-dose treatments of *S. grandiflora* [13,14].

DISCUSSION

The present study was aimed to evaluate the antiarthritic activity of *S. grandiflora* against formaldehyde-induced arthritis in rats by measuring the biochemical parameter, radiological examination in rats. The findings of this study have demonstrated that aqueous extract of *S. grandiflora* is a potent antiarthritic activity against RA.

Formaldehyde-induced paw edema in rats is one of the most suitable test procedures to screen anti-inflammatory and antiarthritic agents. Antiarthritic activity was reported to be mediated either by inhibition of phospholipase-A2 sis of prostaglandins from arachidonic acid and also by blocking the release of vasoactive substances such as histamine, serotonin, and kinins.

The main aim of the study is to reduce the pain, swelling inflammation in the joints using these drugs is to prevent degradation in joints and function restore of disabled joints, several side effects are reported by these drugs side effects such as gastrointestinal disease, immune deficiencies, and humoral disturbances. The RA primary goal is to reduce

Table 4: Effect of *Schisandra grandiflora* on serum C-reactive protein

Days	Control	Induced	Standard	Test 1	Test 2
1	1.16±0.01	1.67±0.01	1.63±0.03	1.63±0.03	1.15±0.02
5	1.19±0.02	1.59±0.02	1.42±0.02	1.59±0.02	1.21±0.02
10	1.23±0.03	1.45±0.02	1.33±0.02	1.17±0.02	1.13±0.02

All values are expressed as mean±standard deviation (n=4), significant at p<0.05*, p<0.01** AQESG: Aqueous extract of *Schisandra grandiflora*

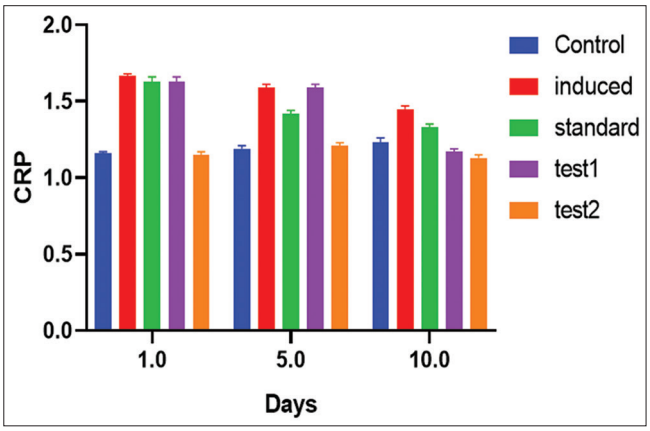

Fig. 5: Graphical representation of serum C-reactive protein levels in rats. **p<0.01 when compared to normal control. **p<0.01 when compared to arthritic control

side effects with therapeutic activity; nowadays, Ayurvedic systems, Siddha, are increased in alternate therapies including arthritis [15-17].

CONCLUSION

The recent study was shown the antiarthritic activity of *S. grandiflora* of plant extract in formaldehyde-induced arthritis in rats. The extracts are evaluated for their antiarthritic activity in formaldehyde-induced arthritis model in rats. A significant antiarthritic activity was noted with plant extracts but relatively more activity with aqueous extract of high dose which can be accounted for difference in phytoconstituents, i.e., terpenoids was presented in this extract.

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