



Leaves of *Stereospermum suaveolens* DC Exhibit Anti-inflammatory and Anti-arthritic Potential Action in Experimental Animals

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Authors' contributions

This work was carried out in collaboration between both authors. Author RRC submitted work is part of Ph.D. research activity. Author DDB has guided and supervised the research work. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The experimental investigation of current research work was to identify traditional rich claim of *Stereospermum suaveolens* DC leaves for anti-inflammatory and anti-arthritic potential action in animals.

Study design: Ethyl acetate fraction of *Stereospermum suaveolens* DC (Bignoniaceae) methanolic extract of leaves evaluated at 125mg/kg, 250mg/kg and 500mg/kg (p.o.) doses for anti-inflammatory and anti-arthritic activity.

Methodology: Ethyl acetate fraction of *Stereospermum suaveolens* DC (Bignoniaceae) methanolic extract of leaves was evaluated for phytochemical investigation for total flavonoid content using UV spectroscopy and TLC study. Carrageenan induced rat paw edema (Acute method) and Freund's complete adjuvant (FCA) induced chronic arthritis in wistar rats were used as an animal models to claim *Stereospermum suaveolens* DC leaves for anti-inflammatory and anti-arthritic potential. The rat paw volume and percentage inhibition of the paw edema were evaluated for anti-inflammatory activity. The assessments of arthritis in rats were measured by haematological values and radiological examinations.

Result: Ethyl acetate fraction of *Stereospermum suaveolens* DC (Bignoniaceae) methanolic extract

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of leaves showed presence of total flavonoids and saponins. The significant inhibition in paw volume and edema ($p < .01$) obtained at 250mg/kg and 500mg/kg oral dose. These obtained results were established confirmation outcome for presence of rich flavonoid contents in *Stereospermum suaveolens* DC leaves and provides valuable source of bioactive phytoconstituents.

Conclusion: Ethyl acetate fraction of *Stereospermum suaveolens* DC (Bignoniaceae) methanolic extract of leaves showed significant inhibition of inflammatory reaction as compared to standard drug indomethacin *Stereospermum Suaveolens* DC leaves were showed potential therapeutic role in treatment of inflammation and arthritis cases.

Keywords: Carrageenan; *Stereospermum suaveolens* DC; flavonoids; thin layer chromatography.

1. INTRODUCTION

Inflammation is protective covering mechanism of the human body to foreign invading agents. It is categorized into acute and chronic type [1]. Acute inflammation is gradual, onset and natural repairing process. It can lead to chronic inflammation on persistently exposure of injurious agents [2]. This chronic inflammation can precipitate tissue and organ failure on long term response. Pharmacological treatment of inflammation is associated with use of non-steroidal anti-inflammatory drugs (NSAIDs), steroids, immunosuppressant and biological agents. The significance and use of NSAIDs, Steroids are restricted due to unavoidable major gastric, renal, cardiovascular and hematological adverse effects [3-4]. However, plant based phytoconstituents can be valuable substitute by considering safety and tolerability on long term consumption in inflammatory diseases. *Stereospermum suaveolens* DC is large medicinal tree found in wild forest and semi evergreen regions of India. It is also native to Bangladesh, Sri-Lanka and Myanmar. It is traditionally known as patala belonging to Bignoniaceae family [5-6]. Bioactive phytoconstituents of *Stereospermum suaveolens* DC identified mainly are lapachol, glycosyloxyflavone, 6-hydroxy luteolin-7-galactoside, p-coumaric acid and tricontanol [7-10]. Extensive literature survey indicates that plant has rich source of phenolic and flavonoid constituents. These phytoconstituents reported as effective Antioxidants, Anti-inflammatory, Antimicrobial agents [2,8,10]. There is need to explore activity of these phytoconstituents to lessen burden and cost of treatment in chronic inflammatory diseases. Various research articles also published on *Stereospermum suaveolens* DC plant that is exhibiting neuroprotective, analgesic, antipyretic activity, antiulcer and gastro protective activity in the stem and bark, hepatoprotective and antioxidant in bark and

roots, anti-diabetic, anti-diarrheal, thrombolytic, antimicrobial activity in the mixture of leaves and stem bark, diuretic activity, anti-inflammatory and anti-arthritis activity, anti obesity, anti-hepato-lipidaemic activity in roots and bark [11-19]. There is no research work is reported on pharmacological activities of *Stereospermum suaveolens* DC leaves for treatment of inflammation and arthritis till date. So considering all gathered information, there is need to perform activity of phytoconstituents of *Stereospermum suaveolens* DC leaves for treatment of inflammation and arthritis conditions.

2. EXPERIMENTAL MATERIALS AND METHODS

2.1 Drugs and Chemicals

Carrageenan, Freund's complete adjuvant (FCA) and Quercetin were obtained from Dolphin pharmacy instruments pvt.ltd, Mumbai, Maharashtra. Analytical grade solvents and chemicals were used for experimentation.

2.2 Plant Material Collection

Stereospermum suaveolens DC leaves collected from periphery of Junner, District: Pune, Maharashtra. The plant specimen were identified and authenticated by expert Dr.P.A.Ingale, Scientist B, Botanical Survey of India, Pune-01. The herbarium specimen no. BSI/WRC/100- 2/Tech/2018/11 was obtained.

2.3 Preparation of Extract and Fractionation

Leaves of *Stereospermum suaveolens* DC were cleaned, washed with water, air dried in shade. The leaves were coarsely powdered in the grinder and stored for experimentation. Leaves powder (150 g) was defatted first by petroleum

ether solvent. Then extraction continued with chloroform and methanol as second and third cycle respectively [20-21]. These extractions were carried out by using Soxhlet apparatus. Concentrated methanolic extract was obtained with the help of Rotary Vacuum Evaporator (Dolphin-RVE/MCPL/2012). The methanolic crude extract of *Stereospermum suaveolens* DC leaves was mixed with ethyl acetate solvent in ration of 1:1. Ethyl acetate fraction of extract was stored for experimentation [21-22].

2.3.1 Phytochemical investigation

The Phytochemical test for obtained fraction was performed to identify flavonoids, alkaloids, glycosides, phenolic and saponins as per standard procedure [21, 23].

2.3.2 Study of thin layer chromatography

Thin layer chromatography a type of liquid chromatography was performed as described by Wagner and Baldt, 1996 [24]. The solvents toluene: ethyl acetate: formic acid (7:3:0.1) were used as mobile phase for separation of sample mixture. The fluorescent flavonoids visualized under UV chamber and compared with Rf values of standard flavonoids [25-27].

2.3.3 Estimation of total flavonoid contents

Aluminium chloride colorimetric method was applied for evaluation of total flavonoid contents [28]. On the basis of the standard calibration curve total flavonoids content (mg/g) determined. The data were expressed as milligram quercetin equivalent. Mean values were calculated [27-28].

2.3.4 UV spectra

UV –Vis spectrophotometer (Shimadzu 3000) study was used for presence of phytoconstituents in sample at 200-400 nm wavelength range [27].

3. SELECTION OF ANIMALS FOR EXPERIMENTS

Wistar rats (150-175g of either sex) and female swiss albino Mice (25-30 g) acquired from NIBS (National Institute of Bioscience), senapati bapat marg, Pune-16, Maharashtra for activity. These animals were maintained under well conditioned animal house at an ambient temperature $25\pm 1^{\circ}\text{C}$ and light-dark (12 h: 12 h) cycle. The approved protocol number was MCP/IAEC/12/2017 by

Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA.

3.1 Oral Acute Toxicity Method

Swiss Albino Mice (25-30g), Ethyl acetate fraction of leaves extract, oral feeding needle and 1 ml tuberculin syringe used for oral toxicity experiment. The procedure was followed as per OECD guidelines no.423. The toxicity parameters were assessed from 24 hrs to 14 days [29]. Selections of dose of Ethyl acetate fraction of *Stereospermum suaveolens* DC Leaves extract were 125mg/kg, 250mg/kg and 500mg/kg finalized for experimentation.

3.2 Experimental Procedure

The wistar rats (n=6) per group maintained for anti-inflammatory and anti-arthritis activity as per followings:

Group 1 (Gr1): Disease control (Carrageenan / FCA induced); Tween 80 5ml/kg/day; orally.

Group 2(Gr2): Standard Indomethacin 10mg/kg/day; orally.

Group 3(Gr3): Test 125mg/kg Dose/ day; orally.

Group 4 (Gr4): Test 250mg/kg Dose /day; orally.

Group 5 (Gr5): Test 500mg/ kg /day; orally.

3.2.1 Acute model of rat paw edema induced by Carrageenan

Oral dose of control group (Tween 80,5ml/kg), standard (Indomethacin, 10mg/kg) group and test (125, 250 and 500mg/kg) groups were carried out initially. After One hour, these groups (Gr1-Gr5) were administered 0.1 ml of 1 % carrageenan solution in sub-plantar region of the rat paw to incite edema. Then at 1,2,3,4 & 5 h interval rat paw volumes were measured by Digital Plethysmometer (VJ-001). Rat paw edema inhibition was calculated by $[1 - (V_t / V_c)] \times 100$. Where, V_t (edema volume in treatment) and V_c (edema volume in control group) considered [29-30].

3.2.2 Induction of chronic arthritis by Freund's complete adjuvant in wistar rats

Activity of Inductions of Chronic Arthritis in rat was carried out by administration of 0.1ml of Freund's complete adjuvant suspension into sub-plantar tissue region of lower left hind rat paw. One hour prior to Freund's complete adjuvant (FCA) administration, control (Tween 80 5ml)

group, standard (Indomethacin-10mg) group and test groups (125mg, 250mg and 500mg) dosing initiated. Then rat paw volumes in all groups (Gr1-Gr5) were measured by using Digital Plethysmometer. Experiment continued up to 28th days by following similar procedure. The rat paw volume measured at 0, 7, 14, 21 and 28 days respectively. Rat blood samples were withdrawn through retro-orbital method at the end of experimentation for evaluation of hematological, biochemical parameters and radiography examination in arthritic wistar rats was carried out by X-ray (dental) unit [31-33].

4. STATISTICAL ANALYSIS

ANOVA (one way analysis of variance) statistical method with post test Dunnett's comparison of all groups with control group was used for determination significant activity in experiments. Graph pad prism 5 software was utilized for calculation and p-value less than < .05 was considered to be statistically significant. (*p < .05, **p < .01 and ***p< .001) when compared with control [32, 34].

5. RESULTS AND DISCUSSION

5.1 Phytochemical Investigation

Phytoconstituents mainly flavonoid, tannis, saponins, carbohydrate and protein identified significantly in ethyl acetate fraction of *Stereospermum suaveolens* DC (Bignoniaceae) leaves extract (as per Table 1). These flavonoids and saponins are valuable phytoconstituents in the pathophysiological corrections of inflammation and arthritis cases [35].

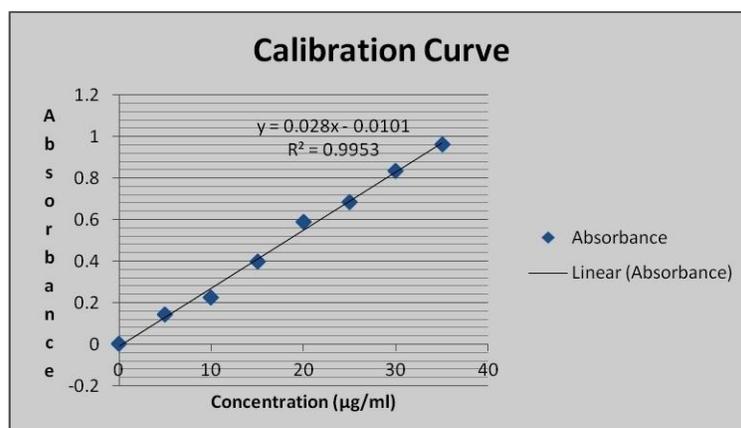
5.2 Estimation of Total Flavonoid Contents

Concentration of 2.964 mcg/ml total flavonoid content was estimated in test sample [27,35]. Equation of calibration curve of standard drug quercetin obtained $y = 0.028x - 0.0101$, $R^2 = 0.9953$. (as per graph 1 calibration curve). This study provided confirmation of flavonoid concentration.

Table 1. Phytochemical analysis of leaves of *Stereospermum suaveolens* DC Fraction

Sr.no.	Chemical test	Methanolic extract	Ethyl acetate fraction
1.	Flavonoids *Test: Lead acetate *Test :Sodium hydroxide	+	+
2.	Tannins *Test: 5% Ferric chloride *Test: Dilute nitric acid	+	+
3.	Saponins * Test :Foam formation	+	+

Note: In this table (+) indicates present for Phytochemical test



Graph 1. Calibration curve showing concentration vs absorbance study

5.3 Study of Thin Layer Chromatography

There were four different spots were identified on TLC plate. Rf (retention factors) calculated and results shown as per Table 2. It was compared with standard flavonoid marker. The mobile phase helped to get separation of components present in the leaves of *Stereospermum Suaveolens* DC sample. The results (as per Figs. 1 and 2) were represented possible presence of flavonoid component in fraction sample (Rf value 0.58).

5.4 UV Spectra Analysis

UV spectra analysis was carried out on UV spectrophotometer (Shimadzu-A114548).

UV absorbance peaks observed in the Table 3 and in the Fig. 3 were evaluated for identification of compounds. Observation peaks at 268 and 280 nm with 0.938, 0.890 absorbance compared for standard quercetin absorbance peaks [27-28]. This spectrophotometric study provided valuable tool for significant presence of flavonoids in the sample.

5.5 Acute Model of Rat Paw Edema Induced by Carrageenan

This method was effective to evaluate anti-oedematous reactions of phytoconstituents in experimental animals. Carrageenan induced edema and paw swelling in rats illustrated release of autacoids or local hormones (histamine, serotonin) and prostaglandins. The significant activity revealed at test-250mg/kg dosing specially at 2- 5 hrs range (as shown in the Table 4). This indicated there was significant suppression of paw swelling and edema (graph 2). Percentage inhibition was high at 2 hr and 5hr at test-250 mg/kg dose obtained (as per Table 5) [11-12,29]. It was confirmed presence of interaction between flavonoids of ethyl acetate fraction of test drug and inflammatory mediators. These flavonoids were inhibited leukocyte migration, release of oxygen free radicals and metabolism of arachidonic acid at injury site.

Table 2. Separation of components with rate of flow (Rf) values for different spots

Sr. no.	Solvent system	Ratio	Number of Spots	Rf values
1	Mobil phase- Solvent Toluene : ethyl acetate :formic acid	7:3:0.1	1	0.28
			2	0.44
			3	0.58
			4	0.84

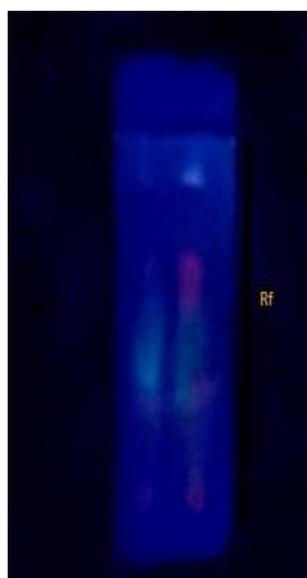


Fig. 1. TLC UV chamber



Fig. 2. TLC (Rf values)

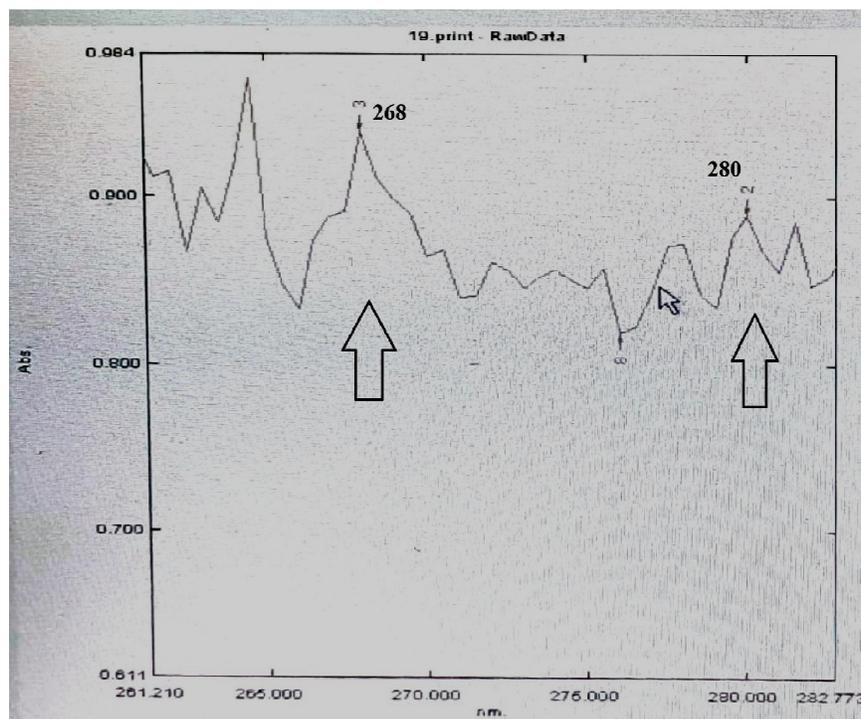


Fig. 3. UV peaks wavelength and absorbance

Table 3. Representing phytoconcentration presence at different wavelength by UV study

Sr. no.	Wavelength nm	Absorbance
1	326	0.668
2	280	0.890
3	268	0.938
4	217	2.910

5.6 Induction of Chronic Arthritis by Freund's Complete Adjuvant in Wistar Rats

Induction of arthritis by Freund's complete adjuvant (FCA) was evaluated for progression of disease, joint inflammation and elevated functional abnormalities in wistar rats. This present study was assessed for various parameters which were indicators to identify severity of arthritis induction. Ethyl acetate fraction of extract of *Stereospermum suaveolens* DC (Bignoniaceae) leaves significantly inhibited arthritis reactions and its progression. Test dose at 250mg/kg and 500mg/kg significant exhibited inhibition of paw edema volume at 7th day and 28th day (as per Table 6 and Graph 3). Ethyl acetate fraction compound of *Stereospermum suaveolens* DC (Bignoniaceae) leaves was

assessed for therapeutic potential in recovery of arthritis condition. It was observed that decreased concentration of white blood cells, significant increased level of red blood cells and hemoglobin level in test treatment when compared with control treated group (as per Table 7). Flavonoids of leaves inhibited appearance of pro-inflammatory enzymes and prostaglandin mediator. This indicated that anti-arthritic potential activity effect of *Stereospermum suaveolens* DC leaves. The decreased level of erythrocyte sedimentation rate provided valuable information in reduction of inflammatory and arthritis reaction. It was significant at 250mg/kg and 500mg/kg dose.

These all parameters were directed to significant anti-inflammatory and anti-arthritic potential of ethyl acetate fraction of *Stereospermum suaveolens* DC leaves extract [31-33].

5.7 Radiographic Study

Radiography examination revealed characteristic involvement of inflammation and arthritis. The paw swelling, redness, pain, swollen tissues, excess thickness of synovium and disability were observed. These inflammatory observations were

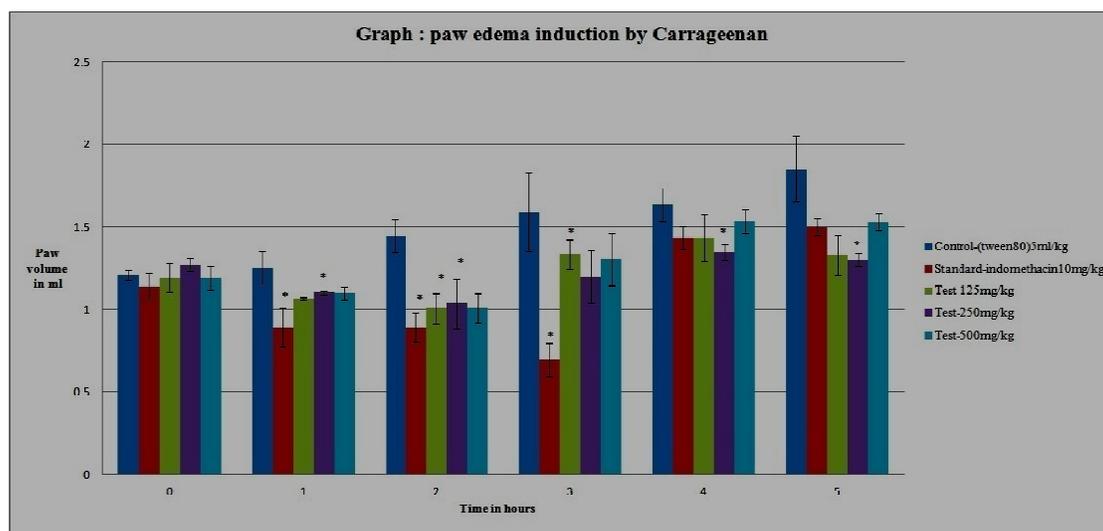
noted extensively in control group animals. Subsequently there were reductions in arthritis and inflammation parameters in standard Indomethacin 10mg/kg group and test treated group. There were significant reductions in parameters like swelling of soft tissues, redness and thickness of synovium at 500mg/kg test dose. The radio graphical study as shown in the Fig. 4 and biochemical parameters proved significant anti arthritic potential of *Stereospermum suaveolens* DC leaves [31-33, 34].

Table 4. Rat Paw edema values

	Time intervals in hrs	Control (Tween 80) 5ml/kg	Standard Indomethacin 10mg/kg	Test- 125mg/kg	Test- 250mg/kg	Test- 500mg/kg
Paw Volume in ml	0	1.21±0.03	1.14±0.08	1.19±0.089	1.27±0.04	1.19±0.068
	1	1.25±0.10	0.89±0.12**	1.065±0.012	1.1±0.014*	1.098±0.04
	2	1.445±0.1	0.89±0.09***	1.005±0.095**	1.035±0.15**	1.007±0.09
	3	1.59±0.24	0.695±0.10***	1.332±0.094*	1.198±0.16	1.302±0.16
	4	1.633±0.1	1.433±0.07	1.433±0.14	1.345±0.05**	1.533±0.07
	5	1.85±0.2	1.50±0.05	1.33±0.12	1.30±0.04**	1.53±0.05

Table 5. Showing percentage of paw edema inhibition

Treatment	Percentage inhibition				
	1h	2 h	3 h	4 h	5 h
Standard-10mg/kg	29	38	57	13	18
Test- 125mg/kg	16	30	17	12	29
Test- 250mg/kg	12	29	26	18	30
Test-500mg/kg	13	31	20	08	18

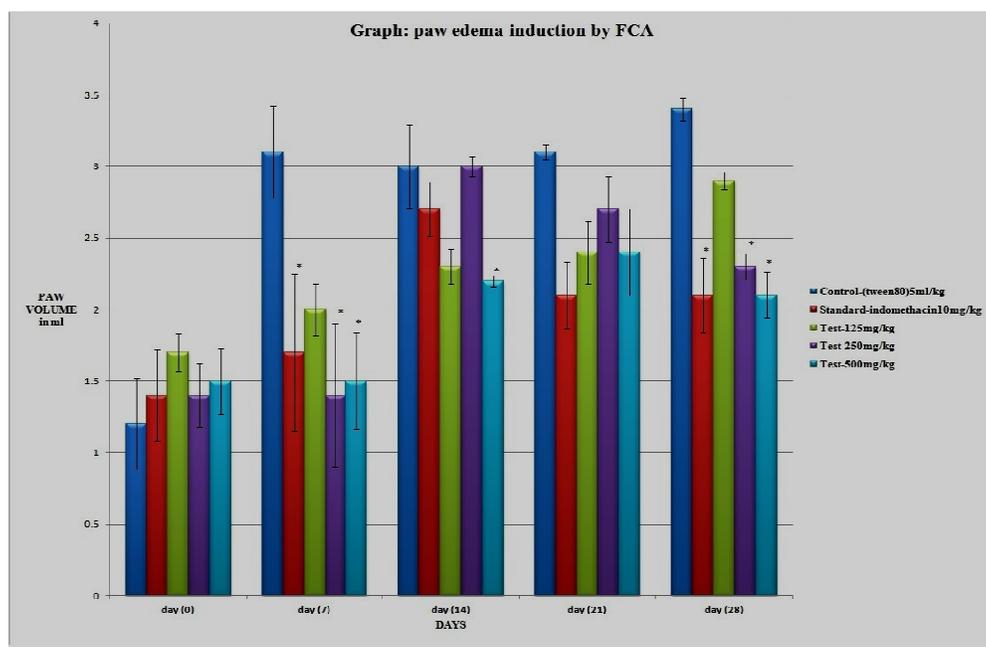


Graph 2. Showing effect of test drug on paw volume by comparing with control group
 Results of Statistical analysis as mean ±SEM, (n=6) by One way analysis of variance (ANOVA) with post test of Dunnett p-value < 0.05 was used as statistically significant. (*p < 0.05, **p < 0.01 and ***p < 0.001, it was compared with control group)

Table 6. Showing observation values of rat paw volume

Groups	Control Tween80 5ml/kg	Indomethacin 10mg/kg	Test- 125mg/kg	Test- 250mg/kg	Test- 500mg/kg
Day-(0)	1.2 ± 0.32	1.4±0.32	1.7±0.13	1.4±0.22	1.5±0.23
Day(7)	3.1±0.32	1.7± 0.55*	2.0±0.18	1.4±0.50*	1.5±0.34*
Day(14)	3.0± 0.29	2.7± 0.19	2.3± 0.12	3.0±0.07	2.2± 0.04*
Day(21)	3.1± 0.049	2.1± 0.23*	2.4±0.22	2.7± 0.23	2.4± 0.3
Day(28)	3.4± 0.085	2.1± 0.26*	2.9± 0.06	2.3± 0.09*	2.1± 0.16*

All tabular and graphical results of Statistical analysis as mean ±SEM, (n=6) by One way analysis of variance (ANOVA) with post test of Dunnett p-value < 0.05 was used as statistically significant. (*P < 0.05, **P < 0.01 and ***P< 0.001, it was compared with control group)



Graph 3. comparing on paw volume of treatment groups with control group in Induction of arthritis

Table 7. Biochemical values in arthritis induction by Freund's Complete Adjuvant

Sr.No.	Control Tween80 5ml/kg	Indomethacin 10mg/kg	Test – 125mg/kg	Test – 250mg/kg	Test – 500mg/kg
WBC thousands /mm ³	40 ±0.08	12±0.2 ***	17±0.23****	12±0.21****	15±0.29****
RBC millions/mm ³	3.9±0.087	6.8±0.4 *	6.9±0.06 **	7.5±0.09 **	8.2±0.16 ***
Hb (mg/dl)	9.6±0.31	14±0.58	13±0.33	14±0.20	16±0.19
ESR (mm/hr)	5.67±0.3	3.83±0.1***	4.13±0.10****	2.00±0.1 ***	2.33±0.11****
SGPT	430±1.8****	411±4.5****	483±12****	440±2.6****	430±3.2****
SGOT	483±2.9	523±1.2	528±1.5	540±0.88	513±1.6
UREA mg/dl	56±0.05	59±0.06	58±0.045	63±0.03	65±0.005
CREATININE mg/dl	1.1±3.5	1.3±1.7	1.1±6.1	1.0±5.2	1.0±5.1

Thin layer chromatography, phytochemical contents in *Stereospermum suaveolens* DC investigation, total flavonoid contents and UV leaves. study were confirmed that presence of flavonoid

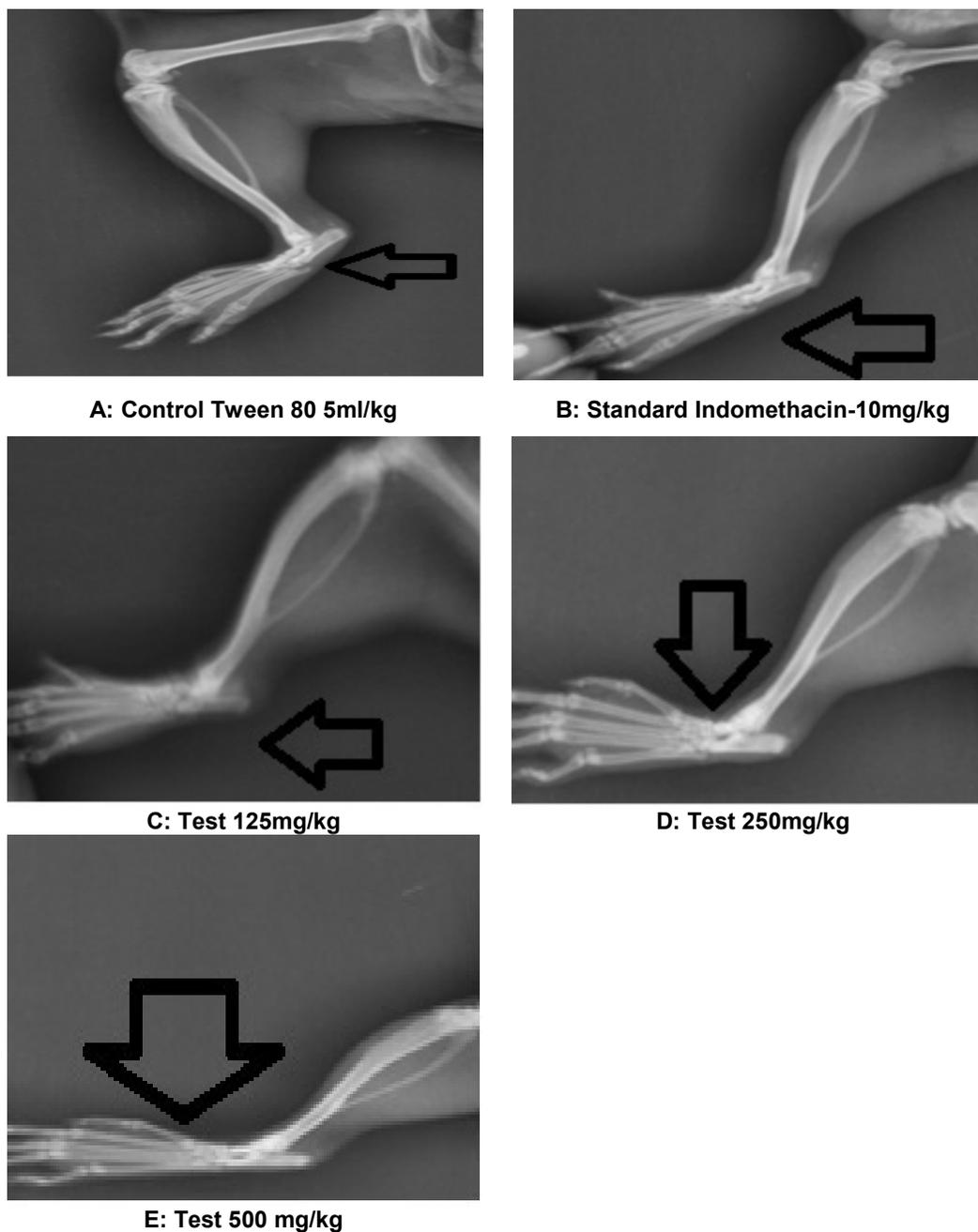


Fig. 4. Radiography Images A-E.
Paw swelling, pain, redness, thickness of synovium and disability of joints decreased in treated groups when images were compared with control group

Therefore, identified phyto groups in *Stereospermum suaveolens* DC leaves can be alternative as herbal drug for existing allopathic medications. It can also improve health condition in acute and chronic inflammatory diseases. Finally It was validated our aim of research work

6. CONCLUSION

From the present study it was concluded that medicinal value of ethyl acetate fraction of *Stereospermum suaveolens* DC leaves in the management of inflammation and arthritis.

It was established traditional claim of leaves for Anti-inflammatory and Anti-arthritic potential in experimental animals. Moreover there is need to explore cellular and molecular mechanism of these flavonoids in chronic disease for betterment of human life.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

CONSENT

It's not applicable.

ETHICAL APPROVAL

The approved protocol number was MCP/IAEC/12/2017 by Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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