Exploring the Anti-inflammatory potential of *Sarcostigma kleinii* Wight & Arn.: An Endemic plant to Western Ghats of India

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ABSTRACT

Conventional approaches addressing chronic & acute pains and inflammations are associated with fewer side effects, and there is a need to explore plant-based medicine to mitigate this issue. *Sarcostigma kleinii* Wight & Arn.is a plant of Western Ghats and not been explored much for its pharmacological and phytochemical profile. Since cellular stress is also involved in the inflammation, various extracts of *Sarcostigma kleinii* Wight & Arn.seeds were screened for total phenolic, total flavonoid content followed by antioxidant studies by DPPH and Nitric oxide free radical assay. The extracts were also screened for both *In vitro* and *In vivo* anti-inflammatory activity. The results suggest that the ethanol extract is moderately safe and exhibited potent inhibition against COX-2 and COX-1 enzymes. The oral administration of the extract was found to significantly reduce the inflammation caused by carrageenan treatment. Compared to the standard Diclofenac the ethanol seed extract effectively reverts the paw edema volume of the inflammatory animals.The phenolic and flavonoid compounds can be interrelated to the antioxidant and anti- inflammatory properties of the plant in the present investigation.

Keywords: *Sarcostigma kleinii* Wight & Arn., Free radical scavenging, COX inhibition, Carrageenan induced rat paw edema model.

INTRODUCTION

Inflammation and pain are common and severe challenges for healthcare professionals. It is increasing the socioeconomic burden [1]. The currently marketed steroids and NSAIDs effectively manage pain and inflammation, with severe side effects limiting them for acute therapy [2]. Since pain and inflammation target all types of people in society, there is a need to address this global problem with safe and effective medicine. It is apparent from the recent literature that plant-based medicine is gaining importance due to its relative safety and chronic usage [3].

Being the source of food and medicine from the ages, plants effectively treat diversified ailments. The secondary metabolites such as flavonoids and other polyphenolic compounds, are proven effective in controlling pain mediation, inflammation cascade, and cellular stress.Herbal products are indispensable in addressing various disease treatments to support the current therapy [4].

Sarcostigma kleinii Wight & Arn.(Icacinaceae) Erumattali in Malayalam,is an unexplored Western Ghat plant with minimum literature on its phytochemistry and pharmacology. Fruits are edible;the bark and leaves treat gastric problems and worm infections. The bark decoction was used for treating intestinal spasms and used to treat gastric ulcers. Externally the plant juice is used for skin diseases and leprosy. The tribals of Kerala are using it for seizures and other mental disorders. The plant needs to be explored for its phytochemical profile and be studied in various pharmacological models [5-7].

MATERIALS AND METHODS

Plant material

The *Sarcostigma* kleinii Wight & Arn.seeds were procured from Kerala Forest areas.

Extraction procedure

The *Sarcostigma kleinii* Wight & Arn. Seeds were powdered after shade drying and extracted with solvents of various polarities such as n-hexane, ethylacetate, ethyl alcohol, and water through Soxhlation. The percentage yield was calculated after the removal of solvent using a rotary evaporator [8].

Preliminary phytochemical investigation

All the extracts of *Sarcostigma kleinii* Wight & Arn.seeds were screened for various phytochemicals through standard procedures [9-10].

Total phenolic content

The Folin-Ciocalteu (FC) method was employed to estimate total phenols in all *Sarcostigma kleinii* Wight & Arn extracts.seeds. Various concentrations of standard Gallic acid were used to construct the calibration curve and repeated with the extracts. Then the total phenolic content in the extracts was estimated by mixing 200µL of the extract solutions, 2.5ml of FC reagent, and 2 ml of Na_2CO_3 solution (7.5% w/v). Absorbance was recorded at 765 nm after 90 min of incubation at 30°C and the values are expressed in terms of mg equivalents of gallic acid [11-12].

Total flavonoid content

Zhishen colorimetric method was used for the assessment of total flavonoid content. Rutin was taken as a reference standard, and a standard curve was plotted against the concentration and absorbance values. For the estimation of total flavonoid content, a solution of extract (125µL) was mixed with 5% Sodium nitrite (75 µL) solution and 10% $AlCl_3$ solution (150 µL) after a few minutes. 1M NaOH (750 µL) was added to this mixture, and distilled water was used to make the volume 2.5ml. This mixture was incubated for 15 min, and when pink color appeared, absorbance was measured in triplicates at 510nm and expressed as gram equivalents of Rutin per gram dry weight [13-14].

***In vitro* antioxidant assay**

DPPH radical scavenging assay:

The extracts of *Sarcostigma kleinii* Wight & Arn.seeds were screened for their ability to neutralize the *insitu* generated DPPH free radicals. 2mL of DPPH solution (0.5mM) was added with 0.2mL of the extract solution and incubated for 20 minutes at room temperature. The absorbance was measured at 515 nm for individual extracts and Ascorbic acid in triplicates. The antioxidant activity was calculated using the formula given below [15].

% Free radical scavenging activity = $[(A_{blank}-A_{sample})/A_{blank}] \times 100$

Nitric oxide radical scavenging assay:

Nitric oxide radical scavenging activity for the extracts was measured using Griess reagent. To sodium nitroprusside (0.5ml) buffered solution, a test solution of various concentrations (1 ml) was added and incubated at 25℃ for 3 hours. This mixture was added to equal volumes of Griess reagent, and 150µl of this solution was transferred to the microplates; absorbance was measured at 540nm using a UV-Visible spectrophotometer, and values are expressed as average values in triplicates [16].

Percentage of NO radical scavenging assay = $[(A_{blank}-A_{sample})/A_{blank}] \times 100$

***In vitro* COX-1 and COX-2 inhibitory assay**

Cyclooxygenase synthesizes prostaglandins (PG) from arachidonic acid, the key targets for NSAIDs in managing acute pain [17]. Cayman COX enzyme kit was used to screen the COX inhibitory activity of the extract. This mechanism-based assay helps to identify the extracts' specificity towards COX-1 and COX-2. The COX enzymes oxidize due to their peroxidase action. The influence of Celecoxib and the extracts in the oxidation of Wurster's blue by COX enzymes into a colored component was measured using a UV-Visible spectrophotometer at 590nm, and IC_{50} values were calculated in µM [18].

Acute toxicity studies

Acute toxicity studies of *Sarcostigma kleinii* Wight & Arn. seed extract was studied using non-pregnant, nulliparous female Wistar rats following OECD-425 guidelines. The first rat was administered with a dose of 175 mg/kg body weight and continued with a factor of 3.2. up to a limit dose of 2,000mg/kg body weight [19].

***In vivo* anti-inflammatory activity**

Carrageenan is a polymer that creates acute and local inflammation in experimental animals. This model is used to screen the effect of natural extracts or compounds for their anti-inflammatory activity. This model triggers a broad range of inflammatory cascades, and the results can be correlated easily with the standard responses [20]. Ethanol seed extract of *Sarcostigma kleinii* Wight & Arn. was selected for the study, and 0.1 ml of carrageenan (1% saline suspension) to the sub-plantar area of the right hind paw of the animal. After the

induction of inflammation, the animals are grouped into four groups as Disease group (under saline treatment), the Positive control group (Diclofenac, 20mg/kg), test groups (extract at 200& 400mg/kg body weight), and non-inflammatory animals taken for the normal group. A plethysmograph was used to measure the edema at 0, 30, 60, 120, and180 minutes of treatment, and the edema volume was calculated.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism TM software, version 6.0 (GraphPad Software, Inc., San Diego, CA, USA), and the values are represented as mean ± SEM. ANOVA (one-way) followed by Dunnet's t-test for multiple comparisons. The level of significance (p value) was considered as less than 0.05.

RESULTS

Preliminary phytochemical screening

The percentage yield and results of the preliminary phytochemical study for all *Sarcostigma kleinii* Wight & Arn extracts.seeds suggest that the plant is rich in secondary metabolites (Table 1&2).

Table 1. Percentage yield

	n-hexane extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Percentage yield	7.86	3.18	5.15	1.34

Table 2. Phytochemical screening of *Sarcostigmakleinii*Wight & Arn.

Phytochemicals	**n-hexane extract**	**Ethyl acetate extract**	**Ethanol extract**	**Aqueous extract**
Alkaloids	-	+	-	-
Glycosides	-	+	+	-
Flavonoids	-	+	+	-
Terpenoids	+	+	+	-
Steroids	+	+	-	-
Tannins	-	-	+	+
Proteins	-	-	+	-
Carbohydrates	-	-	-	+

Amino acids	-	-	+	+
Saponins	-	+	+	+

+ present,– absent

Total phenolic content

Phenolic compounds can participate in redox reactions and give protection against free radical stress. Phenols are important metabolic end products of plants and involve free radical scavenging mechanisms [21]. The total phenolic content of the *Sarcostigma kleinii* Wight & Arn seed extracts was estimated using Folin–Ciocalteu reagent. Ethanolic seed extract of *Sarcostigma kleinii* Wight & Arn.was found to have high phenolic content (42.64±0.29 mgGAE/gm weight). A calibration curve ($Y = 0.0053x + 0.0185$; $R^2 = 0.9983$ was plotted for the gallic acid, and the total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Ethanol extract of *Sarcostigma kleinii* Wight & Arn.was found to have high phenolic content, followed by aqueous extract with 42.64±0.29 & 24.33±1.95 mgGAE/gm weight, respectively (Table 3).

Table 3. Total phenolic content of *Sarcostigma kleinii* Wight & Arn.

Extract	Total phenolic content mg/ml
SCH (*S. kleinii*Wight &Arn.n-hexane extract)	2.34±0.25
SCEA (*S. kleinii*Wight &Arn.ethyl acetate extract)	18.17±0.93
SCE (*S. kleinii*Wight &Arn.ethanol extract)	42.64±0.29
SCAQ (*S. kleinii*Wight &Arn.aqueous extract)	24.33±1.95

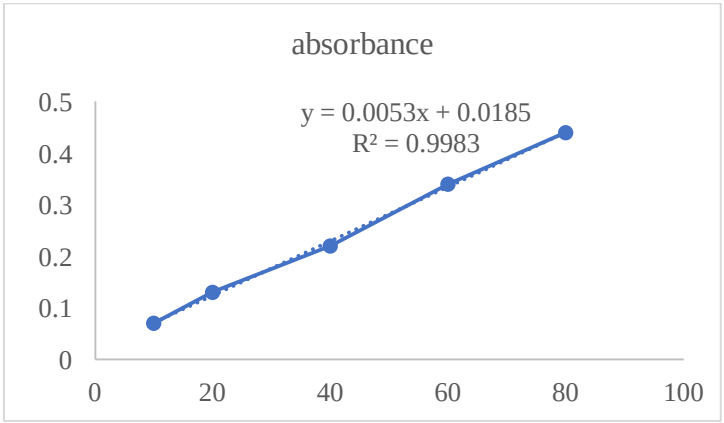

Figure 1. Gallic acid calibration curve

The flavonoid content

The hydrogen-donating ability of the flavonoids makes them an important class of secondary metabolites to fight against cellular stress. Either in free or glycosidic form, Flavonoids can protect against chronic stress and alleviate chronic illness [22-23].The total flavonoid content in the extracts was expressed as Rutin equivalents per gram sample and ethanolic seed extract of *Sarcostigma kleinii*Wight & Arn.was found rich in flavonoids (98.53±1.05 mg/g) followed by ethyl acetate extract (41±1.52 mg/g) (Table 4).

Table 4. Total flavonoid content of *Sarcostigma kleinii* Wight & Arn.

Extract	Total flavonoid content mg/ml
SCH (*S. kleinii*Wight &Arn.n-hexane extract)	1.95±1.06
SCEA (*S. kleinii*Wight &Arn.ethyl acetate extract)	12.03±0.18
SCE (*S. kleinii*Wight &Arn.ethanol extract)	98.53±1.05
SCAQ (*S. kleinii*Wight &Arn.aqueous extract)	41±1.52

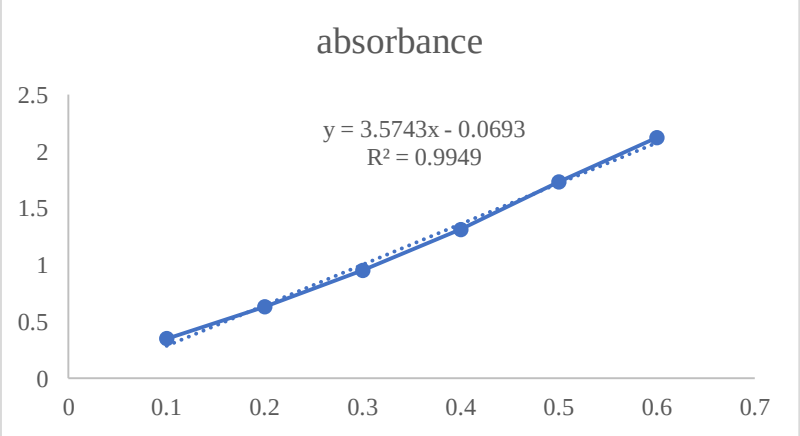

Figure 2. Rutin calibration curve

DPPH radical scavenging assay

The antioxidant activity screening results indicated that the ethanol extract, followed by aqueous and ethyl acetate extracts, has good antioxidant properties in the selected models. The percentage of DPPH radical inhibition property of the extracts was screened from the calibration curve of ascorbic acid, and IC_{50} values were calculated (Table 5 and Figure 4). At 100 µg/mL concentration, *Sarcostigma kleinii* Wight & Arn.ethanol extract with 74.27% of

inhibition (IC_{50}= 44.86 µg/ml) mounted high among the extracts, followed by aqueous extract (65.16; IC_{50}= 59.99 µg/ml) and ethyl acetate extract (56.17; IC_{50}= 92.62 µg/ml)(Figure 5).

Table 5. DPPH assay results of *Sarcostigma kleinii* Wight & Arn.

Concentration µg/mL	Ascorbic acid	n-hexane extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
100	82.11	21.93	56.17	74.27	61.77
75	67.16	14.29	39.17	61.73	57.15
50	58.67	11.37	26.17	54.71	46.42
25	43.48	8.28	20.17	40.15	35.29
IC_{50} µg/ml	**36.66**	**267.8**	**92.62**	**44.86**	**62.05**

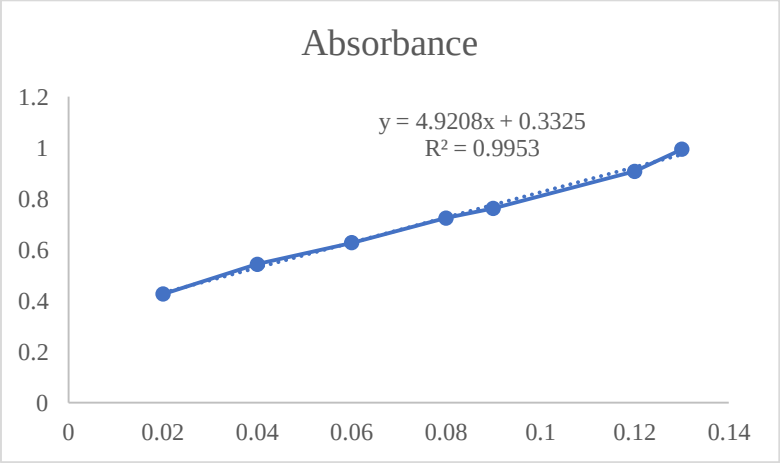

Figure 3. Ascorbic acid Calibration curve

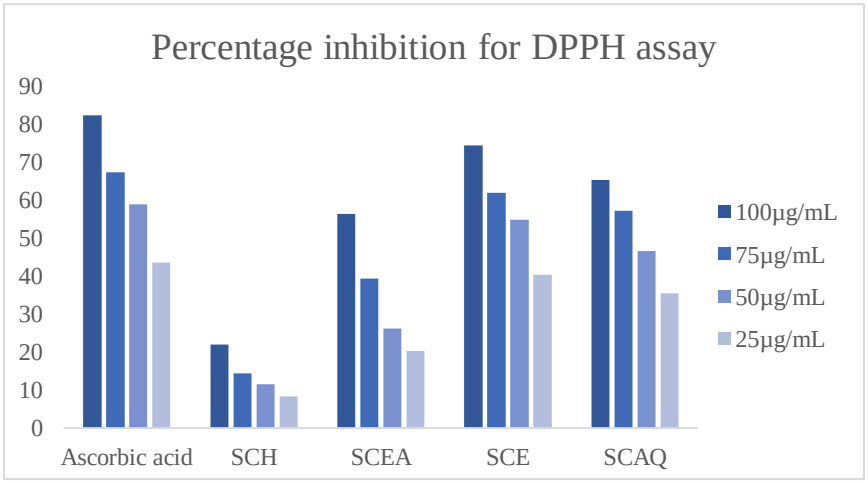

Figure 4. Percentage inhibition of DPPH free radicals

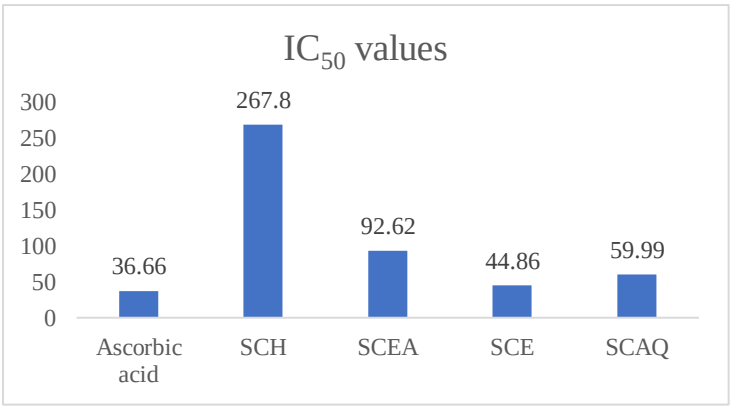

Figure 5. IC_{50} values of extracts for DPPH assay

Nitric oxide (NO) free radical scavenging activity

The ethanol seed extract of *Sarcostigmakleinii* Wight & Arn. inhibited the nitric oxide radicalsat 100 µg/mL of concentration with 74.86% of inhibition with IC_{50} values of 40.5 µg/ml. Ethyl acetateextract inhibited the free radicals by 55.41%, with IC_{50} values of 81.25µg/ml (Table 6).

Table 6. NO free radical scavenging assay results of *Sarcostigma kleinii* Wight & Arn.

Concentration µg/mL	Ascorbic acid	n-hexane extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
100	82.11	25.71	51.78	71.22	61.77
75	67.16	18.92	36.47	59.74	53.66

50	58.67	12.67	24.61	52.67	42.35
25	43.48	8.05	18.34	38.31	31.08
IC_{50} µg/ml	34.34	165.6	81.25	40.5	83.69

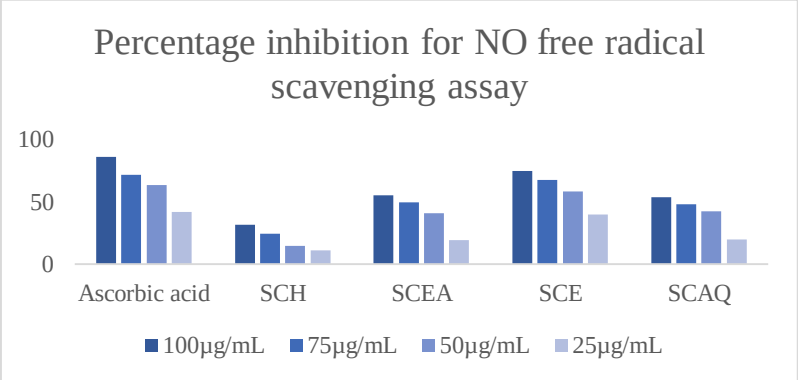

Figure 6. Percentage inhibition of NO free radicals

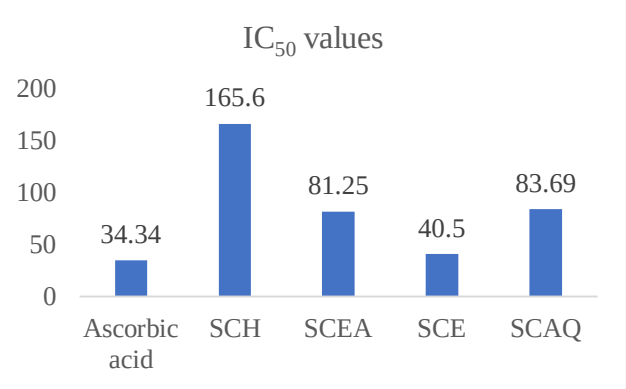

Figure 7. IC_{50} values for NO free radical scavenging assay

COX inhibition assay

COX enzymes are the primary targets for NSAIDs and COX-2 is a better target than COX-1 due to its non-ulcerogenic property [24]. The COX enzyme inhibition assay results revealed that the ethanol extract has specificity and potency toward COX-2 enzyme and is supported by *In vivo* results. The extract of *Sarcostigma kleinii* Wight & Arn.were observed to involve in this mechanism to inhibit the pain mediation, and the percentage of inhibition was high for ethanol extract (Table 7) and are specific towards COX-2 (IC_{50}= 60.5 µg/ml) than COX-1(74.89 µg/ml). Whereas,for COX-1&2, standard celecoxib exhibited IC_{50} values 66.29 &

34.57 µg/ml respectively. Ethanol extract showed potent COX-1inhibition (61.46%) and COX-2 inhibition (67.62%), followed by ethyl acetate extract (42.21%&33.16%) (Figure 8).

Table 7. IC_{50} values of *Sarcostigma kleinii* Wight & Arn. for COX enzyme

Treatment	IC_{50} values (µg/ml)	
	COX-1	**COX-2**
Celecoxib	66.29	34.57
SCH	279.92	473.45
SCEA	118.7	184.66
SCE	74.89	60.5
SCAQ	135.27	161.03

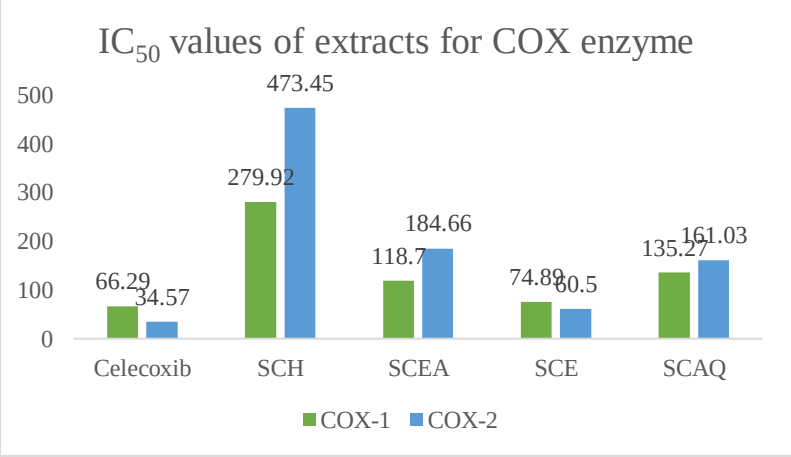

Figure 8. IC_{50} values of extracts for COX enzyme

Oral acute toxicity screening

From the results,it is apparent that the administration of *Sarcostigma kleinii* Wight & Arn.seed extract was safe at 1500 mg/kg body weight without any abnormal clinical symptoms but started showing abnormal behavior in animals at 2000 mg/kg body weight.

***In vivo* anti-inflammatory activity**

Inflammation is a process in the defense mechanism, and it may be life-threatening if it over reacts. Various triggers and pathways are involved in the inflammatory process and can be targeted at different areas of inflammatory progression. Cellular stress can initiate inflammation and develops chronic diseases [25]. Neutralizing excess free radicals is a wise choice to control the inflammation associated with chronic illnesses. Polyphenolic compounds such as flavonoids from plant sources can act as antioxidant agents to protect the cell or tissue from cellular stress [26].

The anti-inflammatory potential of the ethanol seed extract of *Sarcostigma kleinii* Wight & Arn.was estimated by comparing it with Diclofenac. The results suggest that the local inflammation produced by carrageenan injection was significantly reverted after 30 minutes of oral administration of the extracts at 300mg/kg body weight, and the edema volume was decreased from 1.096±0.023 to 0.828±0.023 (Table 8&Figure 9). The anti-inflammatory property of the extract lasted for three hours from the oral administration. The standard Diclofenac reduced paw edema volume from 1.106±0.016 to 0.816±0.015 and was effective from the 30 minutes of administration. Since the ethanol extract is rich in flavonoids and other secondary metabolites may be involved in the reported activities.

Table 8. Effect of *Sarcostigma kleinii* Wight & Arn. on Inflammation

Concentration µg/mL	0 min	30 min	60 min	120 min	180 min
Disease control	1.104±0.015	1.296±0.019	1.326±0.054	1.644±0.025	1.82±0.018
Positive control	1.106±0.016	0.984±0.029*	0.918±0.025*	0.864±0.015*	0.816±0.015*
Low dose (150 mg/kg bw)	1.104±0.023	1.09±0.033	1.002±0.027	0.906±0.035*	0.88±0.023*
High dose (300 mg/kg bw)	1.096±0.023	1.118±0.019	0.92±0.033*	0.908±0.04*	0.828±0.023*

Values are in mean ± SD; (n =6), *p<0.05 was considered statistically significant

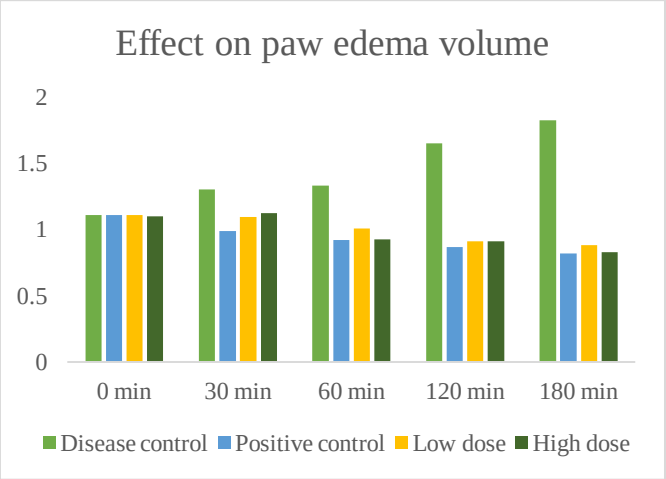

Figure 9. Anti-inflammatory profile of *Sarcostigma kleinii* Wight & Arn.seeds

CONCLUSION

To conclude, *Sarcostigma kleinii* Wight & Arn. seeds are rich in diversified secondary metabolites and polyphenols such as flavonoids. The antioxidant activity screening results indicated that the ethanol extract, followed by aqueous and ethyl acetate extracts, has good antioxidant properties in the selected models. The COX enzyme inhibition assay results revealed that the ethanol extract has specificity and potency toward COX-2 enzyme and is supported by *In vivo* results. The extract was non-toxic at 1500 mg/kg body weight and showed abnormal behavior in animals at the limited dose, i.e., 2000 mg/kg. The ethanol extract reverted the increased edema volume at a 300mg/kg body weight dose, and the values are statistically significant. Since the ethanol extract is rich in flavonoids and other secondary metabolites may be involved in the reported activities.

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CONFLICTS OF INTEREST:

None

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