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ANTIARTHRITIC POTENTIAL OF AQUEOUS AND ETHANOLIC BARK EXTRACTS OF "*SARACA INDICA*" USING FREUND'S ADJUVANT AND COLLAGEN INDUCED ARTHRITIS MODELS.

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Abstract

Saraca indica (Caesalpiniaceae) is a plant, reported for its variety of ethnic medicinal uses and widely grown in Asia, Africa and the Caribbean for its edible bark. The present research work has been planned to investigate the anti arthritic activity of the ethanolic and aqueous bark extract of the plant. Bark powder was successively extracted with ethanol (95%) and water using soxhlet extraction and subjected to phytochemical screening to identify different phytoconstituents. Ld_{50} studies for both (ethanolic and aqueous) extracts were conducted up to the dose level of 2000mg /kg by following OECD guidelines No.425, up and down method. Anti-arthritic activity was investigated using Freund's adjuvant induced arthritis in rats and Collagen induced arthritis model in mice. Preliminary phytochemical studies revealed the presence of sterols, flavonoids saponins tannins in both the ethanolic and aqueous extracts of *S. indica*. No mortality was observed with aqueous and ethanolic extracts up to the maximum dose level of 2000mg/kg. Ethanolic extracts at 200 and 400 mg/kg, shows percentage reduction in paw volume in Freund's adjuvant model the percentage of reduction in paw volume were 47.93% and 52.39% for ethanolic extract and 43.49% and 50.17% for aqueous extract respectively. In collagen induced arthritis models the arthritis index was found 6.18 and 4.12 for ethanolic extract at medium and high dosage. The arthritis index of aqueous extract was found 6.37 and 4.35 at medium and high dosage. In the present study the doses of 200 mg/kg and 400 mg/kg aqueous extract of *S. indica* possesses better anti-arthritic activity since it gives a positive result in reduction in the signs of inflammation in adjuvant induced arthritic and collagen induced arthritis model in rats and mice.

Key words: *Saraca indica,* freund's adjuvant, collagen, anti arthritic.

Introduction

The term "arthritis" used to describe a number of painful conditions of the joints and bones, very often associated with older people, but it can also affect children. About 1:1,000 children develop arthritis, the condition is called as juvenile idiopathic arthritis (JIA). The incidence of RA (Rheumatoid Arthritis) found is 3:10,000 population per year (Kola *et al*., 2018). The prevalence rate is 1% with women affected 3-5 times more than men. It is 4 times more common in smokers than non-smokers (Lemone, 2007). Now a days many ayurvedic practitioners in India are regularly using various native plants for the treatment of different types of arthritis. According to the Indian system of medicine, the medicaments using by the various Ayuervedic practitioners has a profound tradition and a rational background. So it is essential to investigate the rationality of their use in modern scientific terms (Shah *et al*., 2006). *S. indica* bark extracts were previously tested for Astringent to the bowels (Sanjay *et al*., 2016), alexiteric (Yadav *et al.,* 2015), anthelmintic (Singh *et al*., 2014), demulcent (Pradhan *et al*., 2009), emollient (Bhalerao *et al*., 2014), cures dyspepsia, biliousness, colic piles etc. However, the bark extract of *S. indica* has not tested for arthritis. The scientific investigation are essential to prove the potency and to extent their scope for future use. Hence, the aim of the present study is to prove the therapeutic efficacy of the bark as an anti-arthritic agent against, Fromaldehyde induced arthritis, Freund's complete adjuvant (FCA) induced arthritis in rats and collagen induced arthritis in mice.

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Materials and methods

Plant material

Bark of *S. indica* was collected in the month of July

from the Alva Pharmacy, yogaraja arogyadhama, Mizar DK-574225, Mangaluru, India and were dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Preparation of ethanolic extract

The bark powder (750 gm) was packed in a soxhlet apparatus and extracted with one litre ethanol (95%) for 18 h at > 78°C. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into a previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at <50°C. The ethanolic extract of bark of the *S indica* (EEBSI) was finally air dried to remove all traces of solvent and percentage yield of 1%.

Preparation of aqueous extract

About 100 g of bark powder was taken in a round bottom flask (1000 ml) and macerated with 500 ml of distilled water and 10 ml of chloroform (preservative) for 7 days with occasional shaking for every 4 hour in a closed vessel. Then the marc was removed by filtering the extract and then concentrated on a water bath maintained at 50°C and finally air dried thoroughly to remove all traces of the solvent. The aqueous extract of bark of *S. indica* (AQEBSI) was appeared dark brown sticky in nature with percentage yield of 1%.

The two extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air dried sample used for extraction then stored in an air tight containers in a refrigerator below 10°C.

Experimental animals

The Wistar strain albino rats of either sex weighing between 130-180 gm and Albino mice of either sex weighting between 18-23 g were procured from the National Centre for Laboratory Animal Sciences, C/O Sri Venkateswara Enterprises, Bengaluru. All the animals were acclimatized for 7 days after procuring under standard husbandry condition as, 26±2°C room temperature, with relative humidity 45-55% and kept light/ dark cycle for 12:12 h. The animals were fed with synthetic standard diet Amrut Laboratories (Pranava Agro Industries Ltd. Sangli.) Water was allowed *ad libitum* and strict hygienic conditions were maintained. After obtaining prior permission from Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur (Karnataka), all animal studies were performed as per rules and regulations in accordance with the guidelines of CPCSEA (Registration Number 560/02/c/CPCSEA 17th March, 2019.)

Chemicals and drugs

The chemicals used for the antiarthritic study were - Freund's adjuvant [GeNei™Mumbai], Distilled water [Mysore petro chemicals, Raichur,India], Collagen [Sigma Aldrich, Bengaluru], Formaldehyde (Karnataka fine chemicals, Bengaluru, Ibuprofen(S.D. Fine chemicals, Bengaluru), Anaesthetic ether (Sigma Solvents and Pharmaceuticals- Mumbai. All the drugs and chemicals used were of Pharmaceutical grade.

Determination of acute oral toxicity (LD_{50})

The oral acute toxicity study of bark extracts of *S. indica* was determined in female albino mice (18-23 g). Standard husbandry conditions were mentioned. The animals were fasted 4 h prior to the experimentation an Up and Down procedure (OECD Guidelines No. 425) method of CPCSEA was adopted for the acute toxicity studies. Single doses of each extract were administered to the animals and observed for their mortality during 48 h study period (short term toxicity). The LD_{50} studies of the test extracts were conducted up to the maximum dose level of 2000 mg/kg body wt. 1/20th, 1/10th and 1/5th doses of the LD_{50} dose of the individual extracts were selected for the study as low, medium and high doses.

Freund's adjuvant induced arthritis

Male albino rats weighing between (130-180 g) were divided into 9 groups of 6 rats in each(Gerhard *et al.,* 1997). *i.e.* normal control (1% CMC, 1 ml/1kg body weight), toxicant control, standard (Ibuprofen) and six drug treated groups of (ethanolic and aqueous extracts) low, medium and high. All the groups administered with 2% v/v formaldehyde except normal control were injected with single dose of 0.1 ml of Freund's adjuvant and were treated with standard/extract for 12 consecutive days. Paw volumes of both paws were measured plethismographically and body weights are recorded on the 1st day and 21st day of injection. On the days 3, 5, 9, 13 and 21 the volume of injected paw is measured again using plethismograph to note the primary lesion and to study the influence of standard and extracts on this phase. The severity of adjuvant induced disease is followed by measurement of non injected paw (secondary lesions) with a plethismometer. From the day 13th to 21st, the animals are not dosed with the standard/extract. On day 21, the animals were anaesthetized with ether. Blood was collected from the retro orbital puncture later sacrificed by overdose of ether Separated serum was subjected to serum analysis of biochemical parameters. Weight of organs was also noted simultaneously. The edema volume was measured at different time intervals *i.e.* 0, 3, 5, 9, 13

and 21 days. The difference between paw volumes of the treated animals was measured and the mean edema volume was calculated. Percentage reduction in edema volume was calculated by using the formula,

Percentage reduction= $(V_c-V_t/V_c) \times 100$

V_c = Mean volume of paw edema in control group A

V_t = Mean volume of paw edema in drug treated group of animals.

Collagen induced arthritis

Mice weighing between (18-23 g) were divided into 9 groups of 6 mice in each i.e normal control (1% CMC, 1 ml/1kg body weight), toxicant control, standard (Ibuprofen) and six drug treated groups of (aqueous and ethanolic extracts) low, medium and high. All the groups were administered with 0.1 ml of collagen + 0.1 ml of Freund's adjuvant in to base of the tail intradermally for 14 days (Coelho *et al*., 2004; Asano *et al*., 1998). From the day 13th to 21st the animals are not dosed with the standard/extracts, Mice were observed daily for clinical signs of arthritis and each paw was scored on a scale of 0-4 (arthritis index) as follows: 0 = unaffected, 1= 1 type of joint affected, 2 =2 types of joints affected, 3= 3 types of joints affected, 4= 3 types of joints affected and maximal erythema and swelling. The total score for each mice was calculated as an arthritis index[11].On the 21st day all animals were anaesthetized and blood was withdrawn by retro-orbital puncture and collected in plain and EDTA containing tubes, respectively for serum separation. The collected samples were subjected to biochemical evaluation.

Data analysis

In both models the obtained values were expressed as mean ± SD from 6 animals, subjected to statistical analysis by using one way ANOVA followed by Dunnett's-'t' -test to verify significant difference if any among the groups. P<0.05*, 0.01** and 0.001*** was considered significant.

Results and discussion

Ethanolic and aqueous extracts of the bark of *S. indica* were subjected to phytochemical screening It was found that alcoholic extract contained sterols, saponins, flavonoids, carbohydrates and alkaloids whereas aqueous extract contained saponins, flavonoids, carbohydrates and alkaloids.

Acute oral toxicity study

The mice treated with aqueous and alcoholic extract of bark of *S.indica* (AQEBSI and EEBSI) at a dose of 2000 mg/kg, p.o. exhibited normal behavior, without any signs of passivity, stereotypy and vocalization. Their motor activity and secretory signs were also normal and no sign of depression. AQEBSI and EEBSI even up to the dose level of 2000 mg/kg body weight did not produce any behavioral symptoms or mortality. So 1/20th, 1/10th and 1/5th doses of LD_{50} maximum dose tested was selected as low, medium and high doses and were used in the

Table 1: Effect of EEBSI and AQEBSI on Freund's adjuvant induced arthritis in rats at different time intervals.

Treatment	Dose mg/kg p.o.	Paw volume (mean±SEM)						% reduction in oedema volume (mean±SEM)				
		0 day	3 day	5 day	9 day	13 day	21 day	3rd day	5th day	9th day	13th day	21st day
Toxicant control (FA)	0.1ml (SP)	0.82 ±0.01	1.27 ±0.03^{ns}	1.49 ±0.08*	1.61 ±0.01*	1.68 ±0.01*	1.87 ±0.01*	29.0 ±5.19^{ns}	44.75* ±1.13	48.97 ±0.93*	50.94 ±1.26*	55.49 ±0.90*
Standard (Iboprofen)	50	0.80± 0.01	0.95± 0.00**	0.95± 0.00**	0.93± 0.01**	0.90± 0.08**	0.83± 0.01**	25.27± 1.76**	36.30± 0.36**	42.25± 0.82**	46.99± 0.80**	55.02± 1.36**
EEBSI	100	0.82± 0.01	1.24± 0.02*	1.26± 0.01*	1.3± 0.01**	1.28± 0.01*	1.25± 0.02**	2.49± 1.21*	15.05± 0.95*	19.57± 1.38*	23.71± 0.95*	33.28± 1.38*
EEBSI	200	0.85± 0.01	1.19± 0.02**	1.17± 0.01**	1.11± 0.01**	1.05± 0.02**	0.97± 0.01**	6.45± 1.17**	21.20± 1.34**	30.88± 1.78**	37.13± 1.44**	47.93± 1.31**
EEBSI	400	0.8± 0.08	1.0± 0.02**	1.0± 0.01**	0.95± 0.01**	0.93± 0.01**	0.89± 0.01**	20.75± 1.44**	32.91± 1.87**	41.21± 1.06**	44.25± 0.62**	52.39± 1.11**
AQEBSI	100	0.85± 0.01	1.21± 0.03*	1.35± 0.08*	1.46± 0.01**	1.46± 0.01*	1.44± 0.02**	4.52± 1.19*	9.50± 1.34*	9.25± 1.32*	12.80± 1.39*	23.02± 1.51*
AQEBSI	200	0.84± 0.01	1.17± 0.08**	1.2± 0.02**	1.2± 0.03**	1.16± 0.04**	1.05± 0.06**	7.84± 1.70**	18.95± 0.64**	25.75± 0.93**	30.63± 1.44**	43.49± 1.70**
AQEBSI	400	0.83± 0.01	1.15± 0.01**	1.11± 0.02**	1.08± 0.02**	1.0± 0.02**	0.93± 0.01**	8.93± 2.12**	25.13± 1.85**	32.95± 1.92**	40.01± 1.97**	50.17± 1.09**

n=6 Significant at *P*<0.05*, *P*<0.01** and ns-not significant. EEBSI - Ethanolic extract of bark of *S.indica,* AQEBSI - Aqueous extract of bark of *S.indica.* FA- Freund's adjuvant. SP-Subplantar

Table 2: Effect of EEBSI and AQEBSI on biochemical parameters in Freund's adjuvant induced arthritis in rats (mean±SEM).

Groups	Treatment (p.o.)	ALT (U/L)	AST (U/L)	ALP (mg/dL)	BUN (U/L)	CHO (mg/dL)	TRI (mg/dL)	TP (U/L)	GLU (mg/dL)	CRE (U/L)	ALB (U/L)
Normal control	Vehicle (10 ml/kg)	49.50± 0.89	114.07± 3.34	68.76± 2.58	61.30± 0.79	76.46± 4.53	59.48± 7.74	13.26± 1.86	88.59± 0.99	0.80± 0.01	7.25± 0.19
Toxicant control	FA0.1 ml	72.23± 1.14**	264.76± 2.04**	79.31± 3.73**	80.58± 1.80**	167.58± 18.81**	167± 18.90**	6.86± 0.56**	108.86± 8.79**	0.99± 0.03**	4.11± 0.34**
Standard	Ibuprofen 50 mg/kg	56.01± 1.00**	156.4± 11.82**	66.10± 1.06**	73.85± 2.64**	79.01± 1.50**	64.51± 1.78**	10.18± 0.49**	84.14± 7.24**	0.78± 0.01**	5.55± 0.20**
EEBSI	100 mg/kg	68.90± 1.11 ns	256.13± 2.30 ns	77.41± 3.05 ns	63.18± 1.04 ns	160± 16.83 ns	158.45± 6.56 ns	6.30± 1.05 ns	99.35± 1.85 ns	0.92± 0.01 ns	4.16± 0.22 ns
EEBSI	200 mg/kg	62.03± 0.94**	226.53± 2.40**	72.38± 1.65**	68.49± 1.39**	131± 2.59**	96.46± 5.07**	8.41± 0.57**	89.06± 6.23**	0.84± 0.01**	4.85± 0.41**
EEBSI	400 mg/kg	55.11± 0.89**	178.8± 13.86**	69.20± 2.18**	64.13± 1.78**	86.20± 2.29**	68.47± 7.01**	10.25± 0.68**	80.46± 0.62**	0.71± 0.01**	5.01± 0.43**
AQEBSI	100 mg/kg	69.96± 1.03 ns	261.1± 16.50 ns	76.46± 2.09 ns	78.26± 1.49 ns	162± 10.5 ns	160.31± 10.3 ns	6.51± 0.80 ns	104.60± 2.79 ns	0.94± 0.02 ns	4.55± 0.37 ns
AQEBSI	200 mg/kg	64.46± 0.47**	243.11± 1.62**	74.03± 1.21**	69.35± 3.44**	137± 13.6**	106.55± 5.10**	8.87± 0.38**	84.61± 1.89**	0.81± 0.01**	5.37± 0.31**
AQEBSI	400 mg/kg	58.05± 1.35**	191.10± 1.35**	71.70± 1.79**	66.03± 1.63**	89.46± 3.59**	71.70± 5.66**	10.59± 0.69**	82.50± 1.01**	0.74± 0.01**	5.53± 0.29**

n=6 Significant at $P<0.05$*, $P<0.01$** and ns-not significant. EEBSI - Alcoholic extract of bark of *S.indica,* AQEBSI - Aqueous tract of bark of *S.indica.* FA - Freund's adjuvant.

Table 3: Effect of EEBSI and AQEBSI on organ weights in Freund's adjuvant induced arthritis model in rats (mean±SEM).

Groups	Treatment (p.o)	Kidney (g/100g)	Liver (g/100g)	Lungs (g/100g)	Spleen (g/100g)
Normal control	Vehicle (10ml/kg)	1.48 ± 0.02	5.80 ± 0.03	1.44 ± 0.01	0.51 ± 0.02
Toxicant control	FA (0.1ml)	1.23± 0.02**	6.79± 0.04**	1.35± 0.01**	0.78± 0.02**
Standard	Ibuprofen (50mg/kg)	1.43± 0.01**	5.2 ± 0.11**	1.43± 0.01**	0.55± 0.02**
EEBSI	100 mg/kg	1.31± 0.02 ns	5.75± 0.04ns	1.31± 0.01ns	0.68± 0.02ns
EEBSI	200 mg/kg	1.34± 0.01ns	5.73± 0.05ns	1.30 ± 0.01ns	0.63 ± 0.02ns
EEBSI	400 mg/kg	1.39± 0.01*	5.45± 0.01*	1.28 ± 0.01*	0.57 ± 0.02*
AQEBSI	100 mg/kg	1.29± 0.01ns	5.91± 0.04ns	1.36 ± 0.01ns	0.70 ± 0.02ns
AQEBSI	200 mg/kg	1.32± 0.01ns	5.87 ± 0.03ns	1.34 ± 0.01ns	0.66 ± 0.02ns
AQEBSI	400 mg/kg	1.36± 0.03*	5.98± 0.01*	1.32 ± 0.01*	0.60 ± 0.01*

n=6 Significant at $P<0.05$*, $P<0.01$** and ns-not significant. EEBSI - Alcoholic extract of bark of *S.indica,* AQEBSI - Aqueous tract of bark of *S.indica.* FA - Freund's adjuvant.

present study to explore anti-arthritic and anti-ulcer activities.

Effect of Complete Freund's adjuvant induced arthritis in rats

Freund's adjuvant (0.1 ml), when administered at sub plantar region of the paw, is noted with a significant increase in paw volume from 3rd, 5th, 9th, 13th and 21st days of the experimental study. The minimum and maximum percent increase in paw volume is noted on 3rd and 21st day of the experimental study noted as 29.0% and 55.49% respectively.

Standard drug Ibuprofen 10 mg/kg has significantly reduced the raise in paw volume throughout the 21 days of the study period. The percent reductions in paw volume (minimum and maximum) recorded on 3rd and 21st day are 25.27% and 55.02% respectively and a time dependent reduction is observed. The administration of 3 different doses of EEBSI prior to Freund's adjuvant are exhibited with a dose dependent and time dependent reduction in paw volume. The minimum and maximum percent reduction in paw volume recorded with the 3 doses on 3rd and 21st day are 2.49%, 33.28%, 6.45% and 47.93%, 20.75%, 52.39% respectively. Similarly AQEBSI also exhibited a dose dependent and time dependent reduction in paw volume with 3 different doses similar to EEBSI. The minimum and maximum percent reduction in paw volume recorded with 3 doses on 3rd and 21st days of experimental study are noted as 4.52%, 23.02%, 7.84% and 43.49%, 8.93%, 50.17%

respectively. EEBSI exhibited relatively more anti-arthritic activity in this model than AQEBSI and the effect is comparable to the anti-arthritic effect of standard drug Ibuprofen. Results are shown in table 1 and fig. 2.

Estimation of Biochemical parameters in Complete Freund's adjuvant method

The various biochemical parameters include ALT (Alanine transferase), AST (Aspatate amino transferase), ALP (Alanine phosphate), BUN (Blood urea nitrogen), CHO (cholesterol), TG (triglyceride), TP (Total protein), GLU (Glucose), CRE (creatinine), ALB (Albumin), has been investigated for control, standard and both ethanolic and aqueous extracts at low (100 mg/kg), medium (200 mg/kg) and high doses (400 mg/kg). As a result of inflammation induced by adjuvant, the levels of all the biochemical parameters were increased in all arthritis rats as compared to control rats. After extract treatment, the levels of these enzymes were significantly decreased in all groups compared to control rats. Ibuprofen treatment prevented biochemical changes to a greater extent than the aqueous and ethanolic extract of the plant. All the biochemical parameters levels of all the groups were evaluated and compared with each other and the respective results are shown in table 2. EEBSI exhibited relatively more anti-arthritic activity in this model than AQEBSI and the effect is comparable to the anti-arthritic effect of standard drug ibuprofen.

Effect of organ weight in complete Freund's adjuvant induced arthritis in rats

Here the effect on different organs including, kidney, liver, lungs and spleen has been investigated. The organ weight changes have been observed in control, standard and both of the aqueous and ethanolic extracts.

The normal values of organs weights include kidney (1.48 g/100 g), liver (5.80 g/100 g), lungs (1.44 g/100 g) and spleen (0.51 g/100g). In Freund's adjuvant induced arthritis model a decrease in kidney and lungs weight and increase in liver and spleen weights are noted. Standard drug Ibuprofen has significantly contained the change in kidney (1.43 g/100 g), liver (5.20 g/100 g), lung (1.43 g/100 g) and spleen (0.55 g/100 g) weight due to challenge of Freund's adjuvant. EEBSI and AQEBSI with 3 different doses as mentioned above have

Fig. 1: Image of *Saraca indica* plant and bark.

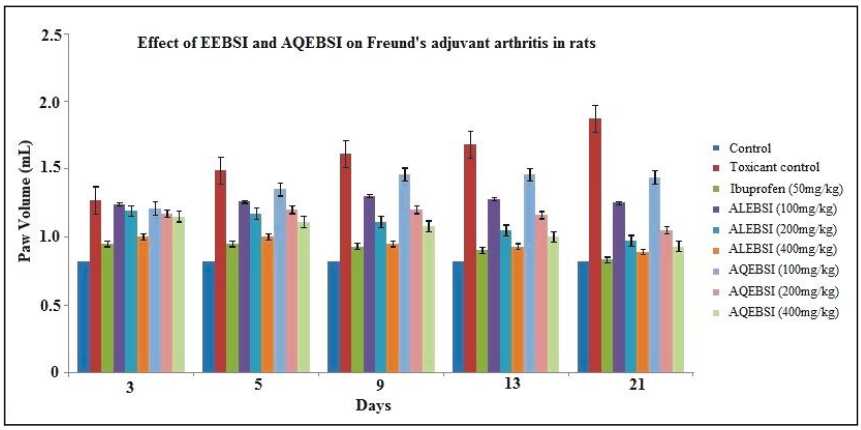

Fig. 2: Effect of EEBSI and AQEBSI on the Freund's adjuvant included arthritis in rats.

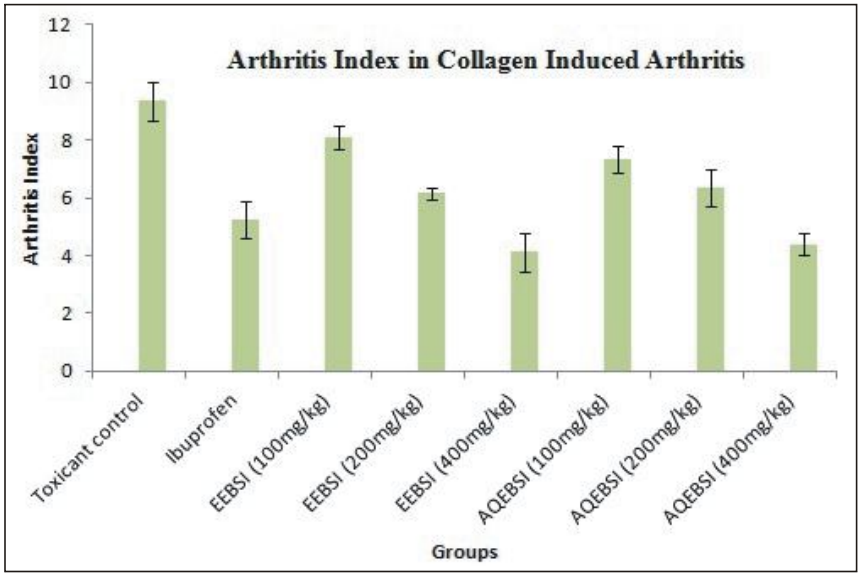

Fig. 3: Arthritis index of collagen Induced Arthritis.

significantly opposed the effect of Freund's adjuvant on weights of the selected organs. A dose dependent anti-arthritic effect is recorded with both the extracts. The values of organ weight noted after treatment with higher doses of both the EEBSI and AQEBSI are liver (5.45; 5.58 g/100 g), lungs (1.28; 1.3 g/100 g), spleen (0.57; 0.66 g/100 g) and kidney (1.39; 1.36 g/100 g) respectively. The respective results are shown in table 3.

Collagen induced arthritis model in mice

Collagen induced arthritis model in toxicant control arthritis index is noted as 7.89 and standard drug Ibuprofen has significantly reduced it to 5.23. The selected extracts EEBSI and AQEBSI with 3 different doses as mentioned

Table 4: Arthritis index of EEBSI and AQEBSI in Collagen induced arthritis in rats (mean±SD).

Group	Treatment	Arthritis index
1	Toxicant control	9.35±0.26**
2	Standard (Ibuprofen 50 mg/kg)	5.23±0.24**
3	EEBSI100 mg/kg	8.12±0.4ns
4	EEBSI100 mg/kg	6.18±0.51*
5	EEBSI400 mg/kg	4.12±0.13**
6	AQEBSI100 mg/kg	7.32±0.37 ns
7	AQEBSI200 mg/kg	6.37±0.22*
8	AQEBSI400 mg/kg	4.35±0.20**

n=6, Significant at p<0.05*, 0.01**and 0.001***, ns = not significant; EEBSI-Ethanolic extract of bark of *S. indica,* AQEBSI- Aqueous extract of bark of *S. indica*

Table 5: Effect of EEBSI and AQEBSI on biochemical parameters in Collagen induced arthritis in mice mean ±SEM.

Groups	Treatment (p.o.)	ALT (U/L)	AST (U/L)	ALP (mg/dL)	BUN (U/L)	CHO (mg/dL)	TG (mg/dL)	TP (U/L)	GLU (mg/dL)	CRE (U/L)	ALB (U/L)
Normal control	Vehicle only 10mg/kg	45.36± 2.35	95.68± 3.48	65.89± 1.89	61.24± 1.98	72.25± 3.23	56.88± 2.62	10.64± 0.45	56.41 ± 1.61	0.29 ± 0.01	4.90± 0.17
Toxicant control	0.1ml collagen +0.1 ml FA intradermally	62.35± 1.05**	287.46± 18.13**	110.60± 8.04**	50.90± 1.81**	104.58± 2.25**	131.64± 5.95**	4.62± 0.47**	109.31± 3.9**	0.42± 0.01**	3.89± 0.07**
Standard Ibuprofen	Ibuprofen 50 mg/kg	49.79± 2.35**	131.4± 3.26**	70.21± 2.87**	69.31± 2.72**	72.40± 3.53**	60.41± 7.92**	9.12± 0.39**	61.27± 2.96**	0.30± 0.01**	5.66± 0.27**
EEBSI	Low dose 100 mg/kg	60.83± 1.79ns	280.75± 15.86 ns	101.45± 3.22ns	49.67± 3.05ns	101.49± 2. 79ns	126.41± 7.31ns	6.13± 0.23ns	101.59± 2.62ns	0.39± 0.01ns	4.11± 0.19ns
EEBSI	Med dose 200 mg/kg	57.82± 2.18*	221.60± 7.34*	84.45± 3.34**	61.31± 2.01**	89.46± 3.36**	100.2± 1.30**	8.14± 0.56**	88.67± 2.20**	0.37± 0.00*	5.28± 0.32*
EEBSI	High dose 400 mg/kg	54.28± 2.13**	170.05± 5.7**	72.61± 3.85**	66.12±3 .22**	81.21± 2.68**	64.74± 2.85**	11.09± 0.34**	73.64± 2.06**	0.33± 0.00**	5.53± 0.35**
AQEBSI	Low dose 100 mg/kg	61.39± 2.62ns	285.08± 14.69 ns	104.28± 3.35ns	50.85± 2.86ns	103.40± 1.4ns	126.35± 4.61ns	6.41± 0.09ns	106.43± 3.47 ns	0.40± 0.00ns	4.23± 0.20 ns
AQEBSI	Med dose 200 mg/kg	59.31± 3.19*	232.4± 11.1**	88.46± 2.99**	63.10± 3.43**	89.60± 16.36**	106.89± 6.49**	8.45± 0.43**	98.67± 3.10**	0.38± 0.01*	4.87± 0.26*
AQEBSI	High dose 400 mg/kg	56.61± 3.91**	179.10± 5.46**	79.54± 4.89**	65.86± 1.70**	86.27± 4.10**	72.88± 3.24**	11.93± 0.56**	70.32± 5.25**	0.37± 0.00**	5.36± 0.30**

n=6 Significant at *P*<0.05*, *P*<0.01** and ns-not significant. EEBSI - Alcoholic extract of bark of *S.indica,* AQEBSI - Aqueous tract of bark of *S.indica.* FA - Freund's adjuvant.

Table 6: Effect of EEBSI and AQEBSI on organ weights in Collagen induced arthritis in rats (mean±SEM).

Groups	Treatment (p.o)	Kidney (g/100g)	Liver (g/100g)	Lungs (g/100g)	Spleen (g/100g)
Normal control	Vehicle (10ml/kg)	0.44±0.02	1.23±0.01	0.26±0.01	0.15±0.007
Toxicant control	0.1ml collagen +FA intradermally	0.41±0.01	1.43±0.01	0.17±0.01	0.08±0.007
Standard	Ibuprofen (50mg/kg)	0.43±0.01**	1.14±0.008**	0.24±0.01**	0.20±0.01**
EEBSI	100 mg/kg	0.35±0.01ns	1.35±0.02ns	0.18±0.005ns	0.16±0.005ns
EEBSI	200 mg/kg	0.38±0.01ns	1.26±0.01ns	0.19±0.006ns	0.17±0.006ns
EEBSI	400 mg/kg	0.39±0.01*	1.20±0.01*	0.21±0.01*	0.18±0.007**
AQEBSI	100 mg/kg	0.35±0.01ns	1.39±0.01ns	0.17±0.01ns	0.13±0.006ns
AQEBSI	200 mg/kg	0.36±0.01ns	1.35±0.01ns	0.18±0.01ns	0.16±0.005ns
AQEBSI	400 mg/kg	0.37±0.01*	1.27±0.02*	0.21±0.01*	0.17±0.008*

n=6 Significant at *P*<0.05*, *P*<0.01** and ns-not significant. EEBSI - Alcoholic extract of bark of *S.indica,* AQEBSI - Aqueous tract of bark of *S.indica.* FA - Freund's adjuvant.

earlier too have significantly reduced arthritis index. The medium and high doses reduced it to 6.18 0%; 4.12% and 6.37; 4.35% respectively. Low doses of both the extracts failed to reduce the arthritis index to significant extent *i.e*., 8.12% and 7.32%. The detail results was shown in table 4 and 5. The arthritis index values were mentioned in table 6 and fig. 3. The various biochemical parameters that has been mentioned in Freund's Adjuvant model has been investigated for control, standard and both ethanolic and aqueous extracts at low (100 mg/kg), medium (200 mg/kg) and high doses (400 mg/kg). The details of the results has been shown in table 5. A dose dependent anti-arthritic effect is recorded with both the extracts. The values of organ weight noted after treatment with higher doses of both the EEBSI and AQEBSI are liver (1.20; 1.27 g/100 g), lungs (0.21; 0.21 g/100 g), spleen (0.18; 0.17g/100 g) and kidney (0.39; 0.37 g/100 g) respectively. The respective results are shown in table 6.

Conclusion

From the empirical evidences it can be concluded that the bark extracts of *S. indica* exhibited significant anti-arthritic activity in experimental animal's rats/mice. A promising anti-arthritic activity was noted with both the extracts but relatively more activity with ethanolic extract which can be accounted for difference in phytoconstituents *i.e.*, sterols as these were presented with ethanolic extract only.

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