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Evaluation Of Analgesic and Anti-Inflammatory Activities of Ethanolic Extract of *Sida Cordata* In Animal Model

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Abstract

Inflammation is a widespread condition that is elicited by the body as a defensive reaction to pain signals. Fortunately, chronic inflammation can contribute to a plethora of illnesses, especially cancer. In folk medicine, *Sida cordata* L. (*Malvaceae*) is used to manage blennorrhea, asthmatic bronchitis, and nasal discomfort. Therefore, the anti-inflammatory, analgesic, and antioxidant activities of ethanolic extract of *Sida cordata* (EESC) were evaluated in animal models by different methods. The hot plate and acetic acid tests were used to evaluate the analgesic activity whereas, carrageen, serotonin, and histamine-induced paw edema and cotton pellet-induced granuloma formation were used to study the anti-inflammatory activity of *Sida cordata*. The administration with 100 and 200 mg/kg of EESC reduced the pain and inflammation indicating that it possesses analgesic and anti-inflammatory activities. The significant anti-inflammatory activity in a dose-dependent manner and the percentage of inhibition at both the doses after 30 min and 6 h was 32.98%, 67.61% and 38.91%, 68.82% respectively, whereas the EESC with a maximum inhibition of 66.94% for serotonin and 53.66% for histamine at the doses of 200 mg/kg. However, in Cotton pellets induced granuloma formation, the EESC at both the doses showed a significant weight reduction, as 43.76% and 51.06%, respectively, and the standard drug showed 55.33% weight reduction of dried cotton pellets. Hence, our study indicates that *Sida cordata* possesses analgesics and anti-inflammatory, activities and it may be useful as an anti-inflammatory agent in inflammation-associated disorders.

Keywords: *Sida cordata,* analgesic, anti-inflammatory, carrageenan, histamine, serotonin, cotton pellet.

Introduction

Indigenous medicinal plants are playing a vital role in replacement therapy for modern medicines owing to side effects (Lipsky 1999 and Gupta 1994). Moreover, the trend of using phytotherapy as alternative medicine has increased the interest nowadays, and therefore, it is a need to develop new agents with more significant anti-inflammatory activities with fewer side effects (Das et al. 1998). Reactive oxygen species have a variety of negative effects; they cause membrane lipids to peroxide, resulting in the formation of lipid peroxides. Protein, amines, and DNA are among the biological substrates that react with lipid peroxides. Modern research has linked the antioxidant properties of numerous plants to oxidative stress protection and a variety of human diseases like cancer, atherosclerosis, aging, inflammation, and certain nervous system disorders (Dhalwal et al. 2005). Hence, there is an increasing interest amongst the researcher those who are doing the inflammatory profile on plants, they also undertaking the antioxidant job.

Sida cordata belongs to the family *Malvaceae*, is a small weed found throughout India, usually on the roadsides and other waste places (Warrier et al. 1996). The plant *Sida cordata* is commonly

known as Bhumibala, Nagabala in Sanskrit; Kurunthotti, Palampasi, Mayirmanikkam in Tamil; Bhuinii in Hindi (Warrier et al. 1996). The literature report states that the *Sida cordata* plant extracts have been used as antibacterial, antitumor, antifungal, antiulcer, anti-tussive, anti-inflammatory, antimalarial, antioxidant, analgesic, antidepressant, anti-hyperglycemic, and hepatoprotective in ayurvedic system of medicine (Gnanasekaran et al. 2012). In our present study, we have evaluated the *in-vivo* analgesic and anti-inflammatory, and *in-vitro* antioxidant activity of the ethanolic extract of *Sida cordata* (EESC) in animal models.

Materials and Methods

Preparation of extract

The powdered root material was evenly packed in the soxhlet apparatus, and continuous hot extraction was carried out for 72 h using ethanol as a solvent. The extract was filtered through Whatman filter paper to remove the impurities, if any, and concentrated by vacuum distillation. Then the concentrated extract was placed in desiccators to remove the excess moisture. The dried extract was kept in an airtight container and used for further studies.

Preliminary Phytochemical Screening

To identify the phytoconstituents, the ethanolic extracts of *Sida cordata* (EESC) were subjected to preliminary phytochemical screening for the presence of various secondary metabolites by using a standard procedure (Harborne 2005).

Screening of Pharmacological activities

Chemical and Reagents

Carrageenan, Diclofenac, Indomethacin, Pentazocinwere procured from Sigma-Aldrich from Roche Pharmaceuticals. All other chemicals and solvents were purchased from SRL Pvt. Ltd., Mumbai, Merck India, Hi-media Pvt. Ltd., Mumbai, and Loba chemicals, Cochin. All the chemicals in this synthesis were of AR and LR grade.

Animals

The Albino rats (150-200 g) were procured from our college animal house and maintained under standard condition as per guidelines of National Institute of Nutrition, India. The study was approved by our institute animal ethical committee Reg.No: 689/Po/Re/03/CPCSEA.

Evaluation of Analgesic Activity

Hot Plate Method

Mice (20-25g) were divided into four groups of six animals in each. Group, I served as normal control and received 0.5%CMC, Group II -served as positive control and received Pentazocin (30mg/kg i.p.), while Group III and IV -received 100 and 200 mg/kg body weight of ethanolic extract of *Sida*

cordata (EESC). The hot plate temperature was kept at 55°±0.5°C, and the cut-off time was the 30s. The basal reaction time in seconds was investigated at 30, 60, and 120 min after the treatment, and the changes if any were noted (Eddy 1953).

Acetic acid induced writhing test in mice

The peripheral analgesic activity of the EESC was determined by the acetic acid-induced writhing inhibition method (Whittle 1964). The Mice were divided into four groups of six animals. Group I- served as normal control and received 0.5% CMC, Group II served as a positive control and received Diclofenac (10 mg/kg, i.p.), while Group III and IV received 100 and 200 mg/kg of EESC. After 1 h oral administration of test drugs, the acetic acid (1%v/v) at a dose of 0.1 ml/10g of body weight was administered intraperitoneally to all the groups. The number of writhing episodes was noted for 10 min and was compared with standard (Suet al 2011).

Evaluation of anti-inflammatory activity(Acute model)

The rats were divided into five groups of six in each. Group I- received 0.5% CMC and served as normal control, Group II- received Indomethacin (10mg/kg) and served as standard, Group III and IV - received the EESC at doses of 100 and 200 mg/kg b.w. orally.

Carrageenan-Induced Paw Edema in Rats- Acute inflammation was produced by the administration of 0.1 ml of 1% w/v Carrageenan in normal saline in the sub plantar aponeurosis of the right hind paw of rats. Drugs were administered 1 h before the injection of Carrageenan. The paw volume was measured at 0 min, 30 min, 1h, 2h, 4h, and 6h after Carrageenan injection (Suleyman et al 1999).

Serotonin and Histamine -Induced Rat Paw Edema - The hind paw edema in the right foot of a rat was induced by previously reported method through the sub plantar injection of 0.1 ml of 1% freshly prepared histamine or serotonin in normal saline (Suleyman et al 1999). The percentage inhibition was calculated by using the formula (Liang Zhu 2011);

$$\text{Percentage inhibition} = 100\ (1 - \frac{\text{Edema volume in treatment}}{\text{(Edema volume in control)}})$$

Cotton Pellets Induced Granuloma in Rats (Chronic model)

The granuloma in rats was induced by implanting cotton pellets (D'Arcy et al 1960). The cotton pellet granuloma model investigates the proliferation phase of inflammation (Winter 1962). The rats were divided into four groups of six in each. The EESC of different doses viz. 100 mg/kg and 200 mg/kg and Indomethacin at 10 mg/kg were administered orally for seven consecutive days. On the eighth day, all the rats were sacrificed, and the cotton pellets covered by the granulomatous tissue were excised and dried in a hot air oven at 60 ^{o}C till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of the cotton pellet on 0 days (before the start of the experiment) from the weight of the cotton pellet on the eighth day. % inhibition=C-T/C x100. Where, T = Test and C= Control.An increment in the dry weight of the pellets was taken as the measure of granuloma formation (Vogel and Vogel 2002).

Statistical analysis

Experimental results are expressed as mean ± SEM. Differences between control and test groups were tested for significance using one-way ANOVA followed by Dunnett's t-test, with $P<0.05$ and $P<0.01$ were considered significant.

Results

Ethanolic extract of *Sida cordata* was subjected to phytochemical analysis and showed the presence of the Flavonoids, Tannins, Saponins, Sterols, Tri-terpenoids, Alkaloids, and Glycosides. The analgesic activity result (Table 1) showed significantly prolonged reaction time to thermal stimuli in mice treated with ethanolic extract of *Sidacordata* (100 mg/kg and 200 mg/kg). Whereas the number of writhing responses showed a significant reduction of 41.31% and 45.93%, respectively, when compared to Pentazocin, which showed 50.43% inhibition at 30mg/kg. However, it showed less analgesic potency than the standard drug Pentazocin (Table 1).

Table 1: Effect of ethanolic extract of *Sidacordata*on hot-plate reaction time and acetic-acidinduced writhing response in mice

Hot-plate reaction time					**Acetic-acid induced writhing response**		
Treatment	**0 min**	**30 min**	**60 min**	**120 min**	**Treatment**	**Writhing**	**% inhibition**
Normal control	2.83 ±0.32	2.67 ± 0.21	2.50 ±0.23	2.38 ±0.22	Normal control (0.5%CMC)	34.62 ± 2.69	-
Pentazocin (30 mg/kg i.p.)	3.00 ±0.26	8.17** ±0.47	10.17** ±0.48	12.67** ±0.49	Diclofenac sodium (10mg/kg)	17.16 ± 1.73	50.43
EESC (100 mg/kg p.o.)	2.83 ±0.20	5.17* ±0.31	7.52* ±0.63	8.54** ±0.43	EESC (100mg/kg)	20.32 ± 1.26	41.31
EESC (200 mg/kg p.o.)	3.17 ±0.31	6.33* ±0.34	8.67** ±0.21	10.83** ±0.48	EESC (200mg/kg)	18.72 ± 1.54	45.93

All values are presented as mean ± SEM, n=6. One way ANOVA followed by Dunnett's test was performed as the test of significance at *p< 0.05, **p< 0.01

The overall anti-inflammatory activities of the ethanolic of*Sida cordata*results were summarized in Table 2. The extract of*Sida cordata*showed the significant anti-inflammatory activity in a dose-dependent manner viz. 100 and 200 mg/kg on Carrageenin-induced model and the percentage of inhibition at 30 min and 6 h ($p<0.01$)were32.98%& 67.61%, and 38.91%& 68.82%,respectively (Table 2).The results were nearly equal to that of the standard drug Indomethacin 69.35% at 10 mg/kg. While the acute edema induced by phlogisticagent's serotonin and histamine exhibited significant activity with a maximum inhibition of 66.94% for serotonin and 53.66% for histamine at 200mg/kg (Table 2).

Table 2: Acute anti-inflammatory activities of ethanolic extract of *Sida cordata*

Treatment	Paw Volume (ml)					
	Carrageenin-induced rat paw edema		Seratonin-induced rat paw edema		Histamine-induced rat paw edema	
	30min	6 h	30 min	6 h	30 min	6 h
Normal control	2.63±0.16	2.74 ±0.18	1.733 ± 0.01	1.74± 0.17	2.941±0.10	2.94±0.12
Negative control	3.83±0.08	4.70±0.08	3.984 ± 0.19	4.14± 0.03	3.328±0.10	4.42 ±0.09
Indome-thacin (10mg/kg)	2.43±0.20 (36.53%)	1.44**±0.06 (69.35%)	1.760±0.58 (55.82%)	1.32**±0.06 (68.10%)	2.631±0.16 (39.21%)	1.42**±0.11 (67.90%)
EESC (100 mg/kg)	2.57±0.16 (32.98%)	1.52*±0.09 (67.61%)	1.977±1.00 (50.38%)	1.48*±0.09 (64.16%)	2.768±0.10 (11.51%)	2.24*±0.14 (46.84%)
EESC (200 mg/kg)	2.34±0.10 (38.91%)	1.46**±0.11 (68.82%)	1.803±0.68 (54.74%)	1.37**±0.11 (66.94%)	2.932±0.20 (36.04%)	1.86**±0.12 (53.66%)

All values are presented as mean ± SEM, n=6. One way ANOVA followed by Dunnett's test was performed as the test of significance at *p< 0.05, **p< 0.01

The cotton pellet-induced granuloma formation is to understand its potential in the sub-acute inflammatory phase. Administration of Indomethacin at 10 mg/kg resulted in 54.58% wet weight reduction, whereas, the EESC at100and 200mg/kg reduced by 38.68% and 50.45% of wet weight, respectively, when compared to a standard drug that reduced to 54.58%. The plant extract at both doses showed a significant reduction in dry weight, at 43.76% and 51.06%, respectively. Indomethacin and plant extracts produced significant anti-inflammatory activity by inhibiting the wet and dry weight of cotton pellets (Table 3).

Table 3: Chronic anti-inflammatory activity of ethanolic extract of *Sida cordata* on Cotton Pellets method

Treatments	Wet Cotton Pellet		Dry Cotton Pellet	
	Weight (mg)	% inhibition	Weight(mg)	% inhibition
Control	216.32 ±2.08	-	56.12 ± 2.64	-
Indomethacin (10mg/kg)	80.46 ±4.09	54.58%	20.14± 1.77	55.33%
EESC (100 mg/kg)	99.52±4.52	38.68%	26.53± 1.54	43.76%
EESC (200 mg/kg)	89.61±3.06	50.45%	24.05± 1.43	51.06%

Discussion

The hotplate method considered being selective to examine compounds acting through the opioid receptor the extract increased mean basal latency, which indicates that it may act via centrally mediated analgesic mechanism (Elisabetsky et al 1995; Pal et al 1999). Acetic acid is believed to cause the peritoneum to produce noxious compounds, leading to a writhing reaction (Bartolini et al 1987). It is a basic, fast, and adaptable model that is ideally suited to assessing the peripheral analgesic activity of any drug (Shinde et al 1999). The acetic acid-induced writhing model represents pain sensation by triggering a localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids (Kumbhare et al., 2011; Ahmed et al 2006). The treatment with *Sida cordata* extract resulted in substantial analgesic action, showing that it has some analgesic effect on both the central and peripheral nervous systems, as shown by decreased pain and reduction of acetic acid-induced writhing.

Inflammation is a well-coordinated reaction to harmful stimuli such as tissue injury and infection (Lumeng and Saltiel 2011). It is triggered to rebuild tissue or the body to its original state. Inflammation is traditionally described as an increase in blood flow, redness of the infected area owing to erythrocyte deposition, and edema (Kiruthiga et al., 2018; Punchard et al 2004). Inflammation causes the release of a variety of cytokines, acute stage proteins, and the transfer of leucocytes to the injured area (Lumeng and Saltiel 2011). Inflammation has been linked to the progression of a variety of diseases in humans, comprising psychiatric, respiratory, gastrointestinal, dental, and renal problems. Ageing, diabetes, obesity, ankylosing spondylitis, multiple sclerosis, pancreatitis, and cancer are all related to inflammation (Lumeng and Saltiel 2011; Lalrinzuali et al 2016). Inflammation-fighting approaches will aid in the reduction of inflammation-related diseases.

Our findings showed that *Sida cordata* may suppress both stages of carrageenan-induced swelling. The impact of the plant extract on serotonin and histamine-induced paw swelling was tested to confirm these findings (Ndebia et al 2011).Histamine is a powerful vasodilator that enhances vascular permeability and is one of the most significant inflammatory mediators (Linardi et al 2002). Given that histamine is the principal mediator in both animals, this research concluded that all dosages of *Sida cordata* efficiently decreased the edoema generated by histamine. *Sida cordata* also decreased serotonin-induced inflammation, suggesting that it may limit the production of mediators implicated in inflammation, such as serotonin, histamine, and prostaglandins (Ndebia et al 2011).

Cotton pellet-induced granuloma formation is considered to be a reliable experimental model for the evaluation of effects on macrophage dysfunction and granuloma formation, maintenance, and progression of granulomas in various disease states. Inflammation is induced in three stages when a cotton pellet is implanted intradermal. The transductive stage, wherein the pellet's wet weight increased, was succeeded by an exudative stage, through which Evans’s blue was released from the circulation around the granuloma cells, and finally, the proliferative stage, wherein the pellet's dry weight increased (Olajide et al 2000). As compared to control, treatment of ethanolic extract at concentration of 200 mg/kg dramatically reduced the development of granulomatous tissue.

Conclusion

Our study demonstrates that the ethanolic extract of *Sida cordata* acts as an analgesic, anti-inflammatory, and antioxidant agent and was investigated using analgesic assessment like acetic acid-induced writhing and hot plate method. Whereas, anti-inflammatory was assessed through carrageenan, serotonin, and histamine-induced paw edema in rats. The analgesic and anti-inflammatory activities of *Sida cordata* may be due to its ability to neutralize free radicals, which are the main players in inflammation. Furthermore, the presence of flavonoids and other polyphenols in *Sida cordata* might explain its analgesic and anti-inflammatory properties. However, further research is needed to clarify their putative molecular mechanisms involved in the combat of inflammation and illness.

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