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EVALUATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *SIDA ACUTA* ON RATS USING BY EDDY'S HOT PLATE METHOD

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ABSTRACT

Evalution of analgesic activity of methanolic extract of *sida acuta* on rats using by eddy's hot plate method ***Method:*** Extraction has been prepared by using ***sida acuata*** leafs, methonal and maceration was done for seven days.The extract was filtered by using muslin cloth.The obtained filtrate was evaporated and cooled to dryness at 45^0 to 55^0c. Until it become like a semisolid. ***Results****:*The animal models employed for screening of analgesic activity in this study were pain-state models using thermal stimuli which include tail-flick and hot plate methods. The methanol extract from the leafs of *Sidaacuta* increase the reaction time of the rats on hot plate method. The difference in the mean reaction time of the extract and the control groups was statistically significant during all observation times. Analgesic effect in Diclofenac on rats was detectable at 45 to 60 min.Hot plate method produces two measureable behavioural components in response to thermal pain, with regard to their reaction times. Responses such as paw licking and jumping in rats are considered to be supraspinally integrated. Thus, the extract to shows these behaviors on hot plate method indicates that it might be acting at supraspinal level. ***Conclusion****:* The methanol extract of the *Sidaacuta* displayed analgesic activity and supported the traditional use of this plant in pain relief. Further study is warranted to identify the active compounds present in this extract and to elucidate the mechanisms involved in its analgesic properties

INTRODUCTION

Higher plants remain as an almost untapped reservoir of potentially useful chemical compounds not only as drugs but also as unique templates that could serve as a starting point for synthetic analogues. Many drugs have been developed with phytochemicals or taking phytochemicals as lead molecules. Some important mainline drugs include digitoxin, aspirin, taxol, ergotamine, morphine, cocaine and reserpine. According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants and it is estimated that at least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. Today nearly 88% of the total global populations turn to plant-derived medicines as first line of defense to maintain health and combating ailments. People of Asia are utilizing plants as part of their routine

health management and oriental world is also coming on the same way. In the direction of developing new antimicrobial agents from herbal resources, research has been geared toward screening of medicinal plants for exploring their antibiotic potential. Many researchers have focused on the investigation of natural products and plant extracts as a source of new bioactive molecules. Sidaacuta (Burm. f.) (Family: Malvaceae) is a taproot and perennial shrub that grow well in many soils. The plant is frequently found in pastures, cultivated lands, roadsides and lawns. It has a variety of traditional uses. In Nicaragua, the decoction of the entire plant is taken orally for asthma, fever, aches and pains, ulcers and as an anti-worm medication; while decoction of the dried entire plant is taken orally for venereal diseases. In India, the hot water extract of the dried entire plant is administered orally as a febrifuge, anabortifacient and diuretic. Previously, antibacterial and antifungal activities of crude extracts of S. acuta have been reported. The aim of the present investigation is to extract and screen flavonoids (free and bound) from different parts (root, stem, leaf and buds) of S. acuta for their analgesic activity.

***Sidaacuta* plant image**

Botanical source: *SidaacutaBurm.*

Family: Malvaceae

Commen Name: Broom Grass, Broomweed, cheeseweed, clockplant, common fan petals, common wire weed, morning mallow, sida southern sida, spiny head sida, spiny headed sida.

Parts used: The whole plant used specially leaves,roots and stem,flowers and fruits.

BOTANICAL CLASSIFICATION:

Kingdom	:	*Plantae*
Sub kingdom	:	*Tracheobionta*
Division	:	*Magnoliophyta*
Super division	:	*Spermatophyta*
Class	:	*Magnoliopsida*
Subclass	:	*Dilleniidae*
Order	:	*Malvales*
Family	:	*Malvaceae*
Sub family	:	*Malvoideae*
Tribe	:	*Malveae*
Genus	:	*Sida*
Species	:	*Acuta*

SYNONYMS:

SidaCarpinifolia L.F

VERNACULAR NAMES:

Telugu : Muttavapulagamu

Hindi : Baraira

Sanskrit : Bala

Marathi : Chikana

Tamil : Palambasi

Oriya : Sunakhodika

Malayalam: Malatanmishiruparuva

English :Broom weed, Broom Grass,Morning Mallow

OTHER SPICIES: *SidaacutaBurm.f, SidaCarpinifoliaL.f*

TRADITIONAL USE: In Malacca, the leaves and roots are boiled and may be used for poutticing the chest to treat coughs. The pounded leaves are used to Promote the healing of wounds and are also used to address influenza, toothaches, chest pains, ulcers,

scabies, abscesses, impotence, gonorrhoea and rheumatism.

MEDICAL VALUES

The roots of *S.cordifolia*known as 'Bala' in Ayurvedic system of medicine are used to treat a variety of ailments including pulmonary tuberculosis, rheumatism, hematuria, urinary and heart diseases. The roots have recently been used to cure Parkinson's disease and as a food supplement for fat loss.(12) It is a tonic, astringent, emollient, aphrodisiac and it is used in the treatment of leucorrhoea, gonorrhea and general debility. Expressed juice of the whole plant is useful for sexual strength and diuretic spermatorrhoea. The juice obtained from the roots is applied to unhealthy sores. Decoction of the roots bark is given in sciatica and rheumatism. The paste of its leaves is applied in ophthalmic diseases. It is also rejuvenative, nutritive and stimulant to the heart. It is especially anabolic to muscle tissues and augments the seminal fluids and promotes reproduction. It also boosts the foetal growth. The plant is analgesic, anti-inflammatory and tonic in nature. It effects on central nervous system and Provides relief from anxiety. It is used to reduce the body weight. It lowers the blood pressure and improves cardiac irregularity. It is used in the popular medicine for the treatment of stomatitis of asthma and nasal congestion Ayurvedic formulations containing *S.cordifolia*should not be prescribed with cardiac glycosides, monoamine oxidase inhibitors and ergot alkaloids. Although no drug interaction have been reported with *S.cordifolia*preparation, but owing to great variation of active constituent, great care should be taken while prescribing *S.cordifolia*with cardiac glycosides (can cause disturbance of heart rhythm), monoamine oxidase inhibitors (as it can potentate the sympathomimetic activity) and ergot alkaloid (can cause hypertension). According to one study a minute dose of *S.cordifolia*given intravenously, causes a sharp and well-marked rise of blood pressure in anaesthetised or decerebrated animals which are maintained for some time.

METHODOLOGY

Plant collection and extraction

- Collection and extraction of leafs
- Leafs of ***sidaacuata*** tree were collected (500gm) and washed with water to clean dust and soil.
- It was weathered inside park overnight and shade dried the flowers.
- Dried materials were taken, crushed it to coarse powder.
- The materials were weighed and transfer in to a clean round bottom flask.
- Sufficient quantity of methanol was added till it totally immersed and maceration it for seven days.
- The extract was filtered by using muslin cloth.
- Mare was pressed and taken out and extraction procedure was repeated two more time with the same mare.
- The obtain filtrate was evaporated and cooled to dryness at 45^0 to 55^0c. Until it become like a semisolid.

PHYTOCHEMICAL ANALYSIS

Methanolic extract of ***sidaacuata***leafs were subjected to qualitative Phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, Phenolic compounds, tannins, proteins and free amino acids, saponins, steroids and terpenoids.

1. Test for alkaloids:

Alkaloids were tested in three extracts, chloroform, ethanol and water. In chloroform extract, 10 mg of chloroform residue was macerated with HCL (2%). The resulting acid solution was filtered and filtrates were separately tested with alkaloidal reagents such as:

Dragendroffs reagent (Potassium bismuth iodide solution) Hager's reagent (A saturated solution of picric acid)

Mayer's reagent (Potassium mercuric iodide solution)

Wagner's reagent (Solution of iodine in potassium iodide)

In alcoholic extract, 10 mg of ethanol residue was macerated with HCI (2%), filtered, Basified with NH_4OH (25%) and extracted with $CHCL_3$. The chloroform soluble portion was evaporated, dissolved in HCL (2%) and tested as in the chloroform extract. In water extract, 10 mg of aqueous residue was dissolved in distilled water (1.5 ml), basified with NH_4OH (25%) and extracted with $CHCL_3$. The chloroform soluble portion was extracted with HCI (10%) and the aqueous acid solution tested as in the chloroform extract.

EXPERIMENTAL WORK:

Animals: A total of 25 Wistar albino rats of either sex weighing 150-250 g were used for hot plate method. All the animals were obtained from the Animal house, Department of Pharmacy,Dr CSN institute of pharmacy, Bhimavaram. All the animals received standard laboratory diet, reverse osmosis water.

PREPARATION OF DRUG SAMPLE: The extract was suspended in distilled water and it is used for the analgesic study.

REFERENCE DRUG: Diclofenac Sodium (10mg/kg) was prepared by dissolving them in normal saline at concentration of 15mg/ml.

EDDY'S HOT PLATE METHOD

The analgesic activity was evaluated by the Eddy's hot plate method. All Formulations for analgesic activity were administered orally. The standard drug Diclofenac sodium was administered in the form of solution in water for injection as vehicle. For the assessment of analgesic activity the animals five were divided into five groups each composed of five animals. All groups received intraperitoneal injection (maximum 1 ml as per ethical norms). The animals were divided into five groups of 5animals each

Group I served as control.

Group II served as standard and was injected Diclofenac sodium (9 mg/kg) intraperitoneal.

Group III served as extract low dose 100 mg/kg and

Group IV served as extract at high dose 200 mg /kg

The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds.

EVALUTION OF ANALGESIC ACTIVITY

Evaluation of analgesic activity of the extract was also carried out using hot plate method. The rats were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. If the reading exceeds 45 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

DISCUSSION

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness. Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain. The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli which include tail-flick and hot plate methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level. While the tail-flick method mediates a spinal reflex to a nociceptive stimulus, hot plate method involves higher brain functions and is regarded a supraspinally organized response.

S.No	Groups	Dose
1	I	Control
2	II	Standard (diclofenac 10 mg/kg)
3	III	Extract low dose 100 mg/kg
4	IV	Extract high dose 200 mg/kg

$$\mathbf{MPA} = \frac{\boldsymbol{reaction\ time\ for\ treatment - reaction\ time\ for\ saline}}{\boldsymbol{45sec - reaction\ time\ for\ saline}} * \mathbf{100}$$

PHYTOCHEMICAL STUDY OF METHANOLIC EXTRACT OF *SIDA ACUTA*

S. No	Class of compound	Plant part (leaf)	Test performed
1	Alkaloids	+	Dragendorff's test, Mayers test
2	Carbohydrates	+	Molish test, Fehling test
3	Glycosides	+	Keller killiani test
4	Phenolic compounds / tannins	+	Ferric chloride test
5	Proteins and amino acids	+	Xantho protein test
6	Flavonoids	+	Ammonia test
7	Saponins	+	With water With Na2CO3
8	Sterols	+	Liebermann-Burchard test, Salkowski reaction, Hesse'sreaction
9	Acid compounds	+	With Na2CO3, With litmus paper
10	Resins	+	With double distilled water, With acetone and conc. HCl
11	Peroxides	-	Potassium Iodide test
12	Polyuronoids	-	Haemotoxylin test

Evaluation of analgesic activity of methanolic extract of sida*acuta* on rats by using Eddy's Hot plate method

Treatments	Reaction Time in Seconds (Mean ± SEM)				
	0 Mins	15 Mins	30 Mins	45 Mins	60 Mins
Control	4.25 ± 0.57	4.50 ± 0.34	4.42 ± 0.45	4.58 ± 0.44	5.17 ± 0.80
Standard Dil^{n}(10mg/kg)	6.50 ± 1.22	11.04±0.73^{*ab}	11.92±0.84^{*ab}	12.83±0.35^{*ab}	13.33±0.83^{*ab}
Extract low(100mg/kg)	4.13 ± 0.54	5.29 ± 0.57	6.75 ± 0.62*^{a}	5.75 ± 0.56	6.79 ±1.24
Extract high(200mg/kg)	6.67 ±0.85^{a}	7.04 ± 0.67^{a}	8.04 ± 0.73^{a}	9.00 ± 0.92^{a}	9.67 ± 0.86

All values by Student's t-test , significant at P <0.005, and SEM = standard error mean . *P<0.05 versus baseline of the respective treatment ^{a}p<0.05treatment versus control,^{b}p<0.05 extract versus morphine sulfate, extract versus sodium salicylate was not significant at all-time points

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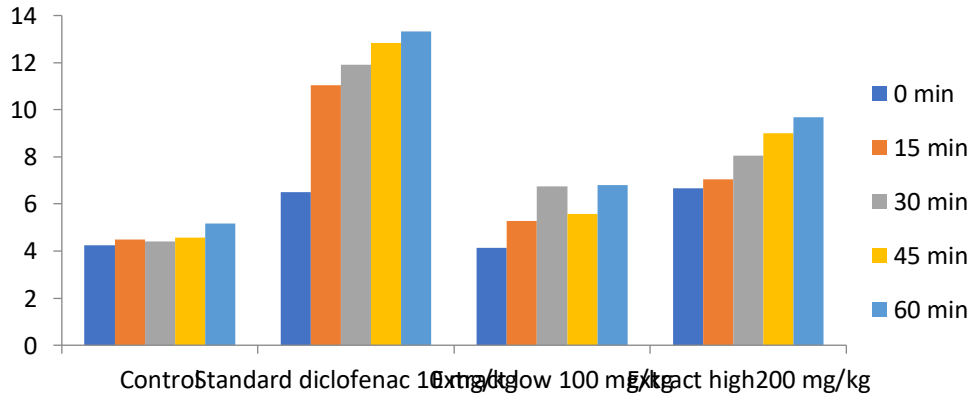

The methanol extract from the leafs of *Sidaacuta* increase the reaction time of the rats on hot plate method in this study. The difference in the mean reaction time of the extract and the control groups was statistically significant during all observation times. Analgesia in Dclofenac treated rats was detectable at 45 and 60 min. Significant analgesic effect was observed between control and the extract tested. Hot plate method produces two measureable behavioural components in response to thermal pain, with regard to their reaction times. Responses such as paw licking and jumping in rats are considered to be supraspinally integrated. Thus, the extract to shows these behaviors on hot plate method indicates that it might be acting at supraspinal level.

CONCLUSION

In conclusion, the methanol extract of the *Sidaacuta* displayed analgesic activity and supported the traditional use of this plant in pain relief. Further study is warranted to identify the active compounds present in this extract and to elucidate the mechanisms involved in its analgesic properties.

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