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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC AND AQUEOUS WHOLE PLANT EXTRACT OF *ASYSTASIA GANGETICA*

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ABSTRACT

In the present study ethanolic and aqueous whole plant extract of *Asystasia gangetica* was investigated for analgesic and anti-inflammatory activity. Analgesic activity was determined by two different methods (tail immersion & hot plate) & anti-inflammatory activity was determined by three different methods (carrageenan, formalin induced paw edema & cotton pellet granuloma) at dose 200 & 400mg/kg b.wt in experimental animals using diclofenac sodium, tramadol, Indomethacin as reference drugs. In all the animals models the results obtained were statistically significant ($P<0.05$) in comparison to control. The results obtained indicate that *Asystasia gangetica* has significant analgesic and anti-inflammatory activities in those animal models.

Key words: *Asystasia gangetica*, analgesic activity, anti-inflammatory activity.

INTRODUCTION

Pain is unpleasant sensation localized to a part of the body. It is both sensation and emotion. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distension or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system [1]. Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms, infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen [2]. Most of the anti-inflammatory drugs are potent inhibitors of Cycloxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema and pain. Hence for treating inflammatory diseases, analgesic and anti-inflammatory agents are required [3]. Many plant products from natural sources are used for the treatment and prevention of diseases. Natural plant compounds are now gaining more pharmacological attention as many unexplored products are showing a wide range of pharmacological activities. *Asystasia gangetica* belongs to the family Acanthaceae. Phytochemical analysis yielded carbohydrates, proteins, alkaloids, tannins, steroidal aglycans, saponins, flavonoids, and triterpenoids. Also yields, minerals: calcium, phosphorus, sodium, manganese,copper, zinc, magnesium, iron. Preliminary phytochemical analysis of hexane, EA, and methanol extracts yielded saponins, reducing sugar, steroids, glycosides, flavonoids and anthraquinones. Therefore the present study was undertaken to investigate analgesic and anti-inflammatory activity of ethanolic and aqueous whole plant extract of *Asystasia gangetica* [4].

MATERIALS AND METHODS

Plant material: The whole plant of *Asystasia gangetica* was collected from Chittoor district,

Andhra Pradesh in the month of Jan-Feb 2015. The plant was authenticated by Dr.K.Madhavachetty, Department of Botany, Sri Venkateswara University Tirupati, and Voucher specimen of the whole plant was kept in museum of SUNRISE UNIVERSITY.

Preparation of ethanolic whole plant extract: The whole plant of *Asystasia gangetica* were shade dried for 3-5 days. Dried plant material was ground to coarse powder using a blender and stored at ambient temperature and passed through sieve and extracted in a soxhlet apparatus for two days using alcohol. The extract was concentrated under reduced pressure using a rotary evaporator. The yield of the extract was found to be 12.5 %. Extract was preserved in a desicator until further use.

Preparation of aqueous whole plant extract: The whole plant of *Asystasia gangetica* were shade dried and powdered. The aqueous extract was prepared by cold maceration for 7days. The powder were soaked in distilled water and stirred intermittently and then left overnight. Macerated whole plant extract were filtered through coarse sieve. The filtrate was dried at reduced pressure in a rotary evaporator and freeze dried. The extracts were used for further studies. The yield of the extract was found to be 13.75%.

Preliminary Phytochemical screening: The presence of various phytochemical constituents in the extract was determined using standard screening tests.

Animals: Wistar rats (150-200g) were used for this study. Before and during the experiment the animals were maintained in well ventilated room at room temperature with natural day-night cycle in polypropylene cages lined with husk in standard environmental conditions temperature 25 ± 2o C, relative humidity 55 ± 10 % and 12:12 light: dark cycle. The rats were fed on standard pellet diet ad libium and had free access to water. The experiments were performed after the approval of protocol by the institution animal ethics committee (IAEC) and were carried out in accordance with current guidelines for the care of laboratory animals.

Acute toxicity studies: Acute oral toxicity studies were performed in rats according to OECD guidelines 425. The dose selected were 200 mg/kg and 400 mg/kg b.wt [5].

ANALGESIC ACTIVITY:

Tail Immersion test: Albino rats of Sprague Dawley strain weighing 170-190 gms of either sex were divided into 10 groups of 5 animals each used. The animals were kept in vertical position to hang the tail up to 5 cm , tail was introduced in hot water at temperature 55± 0.5^0C.The time in seconds to withdraw the tail out of water was taken as the reaction time. The cut-off time, i.e. time of no response was put at 30s.The reaction time was recorded with a stopwatch. The animals were treated with ethanolic & aqueous extracts of *Asystasia gangetica* (200, 400 mg/kg b.wt), saline (vehicle) and standard drug (Tramadol 30mg/kg), were administered intraperitoneally 30 min before the immersion of the tail. The base line latency was measured before and after drug treatment in a regular interval of 0 min, 30 min, 60 min, 90 min and 120 min [6].

Hot Plate method: Albino rats of Sprague Dawley strain weighing 170-190 gms of either sex were divided into 10 groups of 5 animals each. *Asystasia gangetica* ethanolic & aqueous extracts at dose (200mg/kg, 400mg/kg b.wt), saline (control) and Tramadol (30mg/kg) was administered intraperitoneally. Animals in all groups were individually exposed to the hot plate method. Animals were acclimatized to laboratory conditions one hour before the start of the experiment with food and water available as libitium. All drugs were given orally to the respective group rats as suspension in normal saline. Animals were subjected to pretesting on hot plate maintained at 55 ± 0.5^0C.Animals having latency time greater than 15 s on hot plate during pretesting (latency time) were rejected. The reaction time was taken in seconds for forepaw licking or jumping was taken. A cut off time + 10 s was followed avoiding thermal injury to the paws. The reaction time was recorded before and after drug treatment in regular interval of 0 min, 30 min, 60 min, 90 min and 120 min following administration of test or standard drug [7].

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw edema in rats: Albino rats of Sprague Dawley strain weighing 170-200gms of either sex were used. *Asystasia gangetica* ethanolic & alcoholic extracts at dose (200mg/kg, 400mg/kg b.wt), saline (control) and Indomethacin (10 mg/kg) were administered intraperitoneally. After 30 minutes to the above intraperitoneal administration, carrageenan (1% 0.05 ml) was injected subcutaneously in the sub plantar tissue of the right hind paw of each rat. The inflammation was measured using plethysmometer immediately after injection of carrageenan and then 0, 1, 2, 3, 4 and 5h.The average foot swelling in drug treated animals as well as standard was compared with that of control [8] .

Formalin Induced Paw edema in rats: The formalin induced paw edema in rats was done according to the reported method. In this method, 20µL of 2.5% formalin was injected into the subcutaneous tissue of the plantar surface of the left hind paw of rats 1 hour after administration of drugs. The paw volume was determined as per the reported literature [9].

Cotton pellet induced granuloma: Albino rats of Sprague Dawley strain weighing 150-190gms of either sex were used. The animals received ethanolic & aqueous extracts of *Asystasia gangetica* (200, 400mg/kg b.wt), Diclofenac (10mg/kg), saline (control) orally once a day through an oral cannula over seven consecutive days. Sub acute inflammation was produced by cotton pellet granuloma model in rats, on day 1, with aseptic precautions sterile cotton pellets (50± 1 mg) were implanted subcutaneously, along the flanks of axillae and groins bilaterally under ether anesthesia. The animals were sacrificed on the 8th day. The granulation tissue with cotton pellet was dried at 60^{0}C overnight and then the dry weight was taken. Weight of the cotton pellet before implantation was subtracted from weight of the dissected dried pellets. Only dry weight of the granuloma formed was used for statistical analysis [10].

Table-1: Analgesic activity of Asystasia gangetica using tail immersion method in rats

Groups	Dose mg/kg b.wt	Reaction Time (Seconds)				
		0 min	30 min	60 min	90 min	120 min
Control (Saline water)	10 ml	2.21 ± 0.158	2.78 ± 0.342	2.90 ± 0.008	2.80 ± 0.093	2.70 ± 0.008
Standard (Tramadol)	30 mg	4.52 ± 0.093**	5.81 ± 0.082**	5.92 ± 0.119**	5.36± 0.184**	5.22 ± 0.119**
Asystasia gangetica Ethanolic extract	200	2.30 ± 0.049*	3.58 ± 0.172*	3.96 ± 0.363*	3.60 ± 0.180*	3.56 ± 0.363*
Asystasia gangetica Ethanolic extract	400	2.40 ± 0.223ns	3.22 ± 0.409ns	3.34± 0.163**	3.20 ± 0.100ns	3.14± 0.163**
Asystasia gangetica Aqueous extract	200	2.49 ± 0.071ns	3.35 ± 0.057ns	3.78 ± 0.114**	3.76 ± 0.174*	3.58 ± 0.114**
Asystasia gangetica Aqueous extract	400	2.78 ± 0.143*	3.18± 0.273**	3.76± 0.191*	3.60 ± 0.283*	3.76± 0.191*

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table-2: Analgesic activity of Asystasia gangetica by hot plate method in rats

Groups	Dose mg/kg b.wt	Reaction Time (Seconds)				
		0 min	30 min	60 min	90 min	120 min
Control (Saline water)	10 ml	7.31 ± 0.158	8.78 ± 0.342	9.20 ± 0.008	9.80 ± 0.093	9.20 ± 0.008
Standard (Tramadol)	30 mg	9.52 ± 0.093**	12.81 ± 0.082**	14.92 ± 0.119**	15.36± 0.184**	16.92 ± 0.119**
Asystasia gangetica Ethanolic extract	200	8.30 ± 0.049*	9.78 ± 0.172*	11.96 ± 0.363*	12.60 ± 0.180*	13.76 ± 0.363*
Asystasia gangetica Ethanolic extract	400	8.40 ± 0.223ns	9.22 ± 0.409ns	10.34± 0.163**	11.20 ± 0.100ns	12.34± 0.163**
Asystasia gangetica Aqueous extract	200	9.49 ± 0.071ns	10.35 ± 0.057ns	11.78 ± 0.114**	12.76 ± 0.174*	13.88 ± 0.114**
Asystasia gangetica Aqueous extract	400	8.78 ± 0.143*	9.18± 0.273**	10.76± 0.191*	12.60 ± 0.283*	13.96± 0.191*

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table-3: Anti-inflammatory activity of Asystasia gangetica on paw edema induced by Carrageenan in rats

	Dose mg/kg b.wt	**Change in Paw Volume mL**					
		0h	**1h**	**2h**	**3h**	**4h**	**5h**
Control (Saline water)	10 ml	0.62 ± 0.095	0.75± 0.164	0.96± 0.151	1.6 ± 0.300	1.7± 0.397	1.9 ± 0.528
Standard (Indomethacin)	10 mg	0.59 ± 0.003*	0.27 ± 0.024**	0.29 ± .007**	0.60±0.008***	0.44±0 .004**	0.48 ± 0.004**
Asystasia gangetica Ethanolic extract	200	0. 58 ± 0.092^{ns}	0.70 ± 0.123^{ns}	0.82 ± 0.104*	0.75± 0.105^{ns}	0.90± 0.106*	0.95± 0.125^{ns}
Asystasia gangetica Ethanolic extract	400	0.60 ± 0.146^{ns}	0.68± 0.120*	0. 80± 0.055*	0.70± 0.120**	0.82 ± 0.111*	0.97± 0.129*
Asystasia gangetica Aqueous extract	200	0.56 ± 0.102^{ns}	0.66 ± 0.084**	0.77 ± 0.119*	0.68 ± 0.065**	0.79 ± 0.070^{ns}	0.93 ± 0.006*
Asystasia gangetica Aqueous extract	400	0.58 ± 0.050*	0.62 ± 0.100ns	0.75 ± 0.065*	0.64± 0.059*	0.77 ± 0.061**	0.90 ± 0.015^{ns}

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table-4: Anti-inflammatory activity of Asystasia gangetica on paw edema induced by formalin in rats

Groups	**Dose mg/kg b.wt**	**Change in Paw Volume in mL**					
		0h	**1h**	**2h**	**3h**	**4h**	**5h**
Control (saline water)	10 ml	0.89 ± 0.015	1.32± 0.011	1.36± 0.027	1.30 ± 0.026	1.29± 0.027	1.20±0.004
Standard (Indomethacin)	10 mg	0.86 ± 0.023*	1.05 ±0 .021*	1.18 ± 0.048**	1.08±0.040***	1.00± 0.036**	0.50±0.003
Asystasia gangetica Ethanolic extract	200	0. 88±0.022^{ns}	1.30 ±0.015^{ns}	1.37 ± 0.013^{ns}	1.21±0. 025**	1.12± 0.033*	0.72±0.002
Asystasia gangetica Ethanolic extract	400	0.85 ± 0.023*	1.26 ±0.027*	1.34± 0.034*	1.18±0.057*	1.07 ± 0.065*	0.56±0.002
Asystasia gangetica Aqueous extract	200	0. 87 ±0.021^{ns}	1.29± 0.023^{ns}	1.30 ± 0.044*	1.27± 0.015*	1.20± 0.026*	0.75±0.002
Asystasia gangetica Aqueous extract	400	0.86± 0.021^{ns}	1.29 ± 0.032^{ns}	1.28± 0.022*	1.24± 0.034*	1.22± 0.015**	0.55±0.001

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table-5: Anti- inflammatory of Asystasia gangetica on cotton pellet induced granuloma in rats

Groups	**Dose mg/kg b.wt**	**Weight of dry cotton pellet Granuloma(mg)**
Control (saline water)	10 ml	208±3.250
Standard (Diclofenac)	10 mg	13.9±1.126**
Asystasia gangetica Ethanolic extract	200	130±4.764 ns
Asystasia gangetica Ethanolic extract	400	98±4.389**
Asystasia gangetica Aqueous extract	200	142±2.971 ns
Asystasia gangetica Aqueous extract	400	90±2.236**

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control, ns= non-significant.

RESULTS

Acute toxicity studies: The whole plant extract of *Asystasia gangetica* were evaluated for acute toxicity in mice and rats by intraperitoneal and oral administration of extract. No mortality and behavioral changes were observed up to 2 weeks. The ethanolic & aqueous extracts were safe upto 2000mg/kg body weight dose. Based on this test *Asystasia gangetica* was tested at 200, 400 mg/kg body weight for this experiment.

Phytochemical screening: The ethanolic & aqueous extract of *Asystasia gangetica* showed the presence of saponins, steroidal saponins, triterpenoids, carbohydrates, flavanoids, proteins and amino acids.

Analgesic Activity

Tail immersion method in mice: Results of analgesic activity of *Asystasia gangetica* ethanolic and aqueous whole plant extract measured by tail immersion method are given in Table-1. At dose 200, 400 mg/kg *Asystasia gangetica* extract exhibited 50 % inhibition when compared to control, whereas the positive control exhibited 93 % inhibition. From table-1, it is evident that both extracts showed moderate analgesic activity when compared to that of Tramadol.

***Hot plate method in rats*:** Results of analgesic activity of *Asystasia gangetica* ethanolic and aqueous whole plant extracts measured by hot plate method are given in Table-2. The results of the hot plate test revealed that the most significant latency time was observed at dose 400 mg/kg for aqueous extract and the percentage inhibition was found to be 51.73%, when compared to ethanolic extract which was found to be 49.56%, whereas Tramadol showed 83.91% inhibition when compared to control.

Anti-inflammatory Activity:

Carrageenan- Induced paw edema in rats: Results of anti-inflammatory activity of *Asystasia gangetica* ethanolic and aqueous whole plant extracts are given in Table-3. Injection of Carrageenan was done 1h after oral administration of the extract (200,400mg/kg b.wt), Indomethacin (reference drug). Both the ethanolic and aqueous whole plant extracts showed significant inhibition of paw edema at 3h. Ethanolic extract showed 56.25%, whereas aqueous extract showed 60% at dose 400mg/kg when compared to control. Indomethacin showed inhibition of paw edema with a maximum effect of 74.73%.

***Formalin induced paw edema in rats*:** Results of anti-inflammatory activity of *Asystasia gangetica* ethanolic and aqueous whole plant extracts are given in Table-4. Inflammatory edema induced by formalin was significantly inhibited in a dose dependant manner and significant inhibition of edema started at 3hr and significant up to 5th hr. Ethanolic extract showed 53.33% inhibition, whereas aqueous extract showed 54.16% when compared to control. Indomethacin showed inhibition of paw edema with a maximum effect of 86.76%.

Cotton pellet granuloma in rats: Results of anti-inflammatory activity of *Asystasia gangetica* ethanolic and aqueous whole plant extract are given in Table-5. The whole plant extract exhibited a significant and dose related inhibition of the dried weight of the cotton pellet granuloma. The inhibitory values for 200 and 400 mg/kg of ethanolic and aqueous extract exhibited 37.5%, 52.88%, 31.73%, 56.73% respectively. Diclofenac (reference drug) inhibited granuloma tissue formation with a value of 93.31%.

DISCUSSION

In the present study analgesic activity of *Asystasia gangetica* ethanolic and aqueous whole plant extract were screened by two different methods (tail immersion & hot plate). Anti-inflammatory activity was determined by three different methods (carrageenan, formalin induced paw edema & cotton pellet granuloma). Both the activities were determined at dose levels 200, 400 mg/kg b.wt. Diclofenac sodium, Tramadol, Indomethacin was used as standard reference drugs. Central analgesic effects of drugs was determined by tail immersion method, analgesic effect through thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomenon. All the extract increased basal latency probably by acting through centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain while nonsteroidal anti-inflammatory drugs inhibit only peripheral pain [11].
The analgesic effect of *Asystasia gangetica* ethanolic and aqueous whole plant extract was screened using eddy's hot plate method. This animal model shows marked central analgesic effect. Thermal test was selected because of several advantages including sensitivity to strong analgesics and limited tissue damage. All the extract showed significant latency time.

Anti-inflammatory effect was evaluated in the acute phase of inflammation and chronic phase of inflammation. Carrageenan was selected because of its sensitivity in detecting orally acting anti-

inflammatory agents in the acute phase of inflammation [12]. The cotton pellet granuloma method is a model of chronic inflammation and the dry weight has been shown to correlate with the amount of granulomatous tissue formed. Carrageenan induced edema is well established model and is believed to be biphasic. The initial phase has been known (1-2h) to be induced due to the action of mediators such as histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandins release and mediated by bradykinin, leukotrienes, polymorhonuclear cells and prostaglandins produced by tissue macrophages. All the extract showed significant inhibition of paw edema induced by carrageenan and histamine by inhibition of Cycloxygenase synthesis. The cotton pellet granuloma method has been widely used to evaluate transudative, exudative and proliferative components of chronic inflammation [13], because the dried weight of the pellets correlates with the amount of granulomatous tissue, all the extract showed dose-dependent inhibition of granuloma formation in mice.

CONCLUSION

The present study of *Asystasia gangetica* ethanolic and aqueous whole plant extract showed potent analgesic and anti-inflammatory activity. The activity may be due to the presence of chemical constituents mainly flavonoids, saponins that are present as chemical constituents in these extract. Flavanoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitor effects on enzymes involved in the production of the chemical mediator of inflammation. The presence of flavonoids & saponins may be responsible for analgesic and anti-inflammatory activity, further investigation are required to isolate the active constituents and to know the possible mechanism of action of the plant extract.

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