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Analgesic and anti-inflammatory activity of ethanolic and aqueous flower extract of *Sterculia foetida*

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ABSTRACT

Background: In the present study, analgesic and anti-inflammatory activity for ethanolic and aqueous flower extract of *Sterculia foetida* was investigated.

Material and methods: Analgesic activity was determined by two methods (tail immersion & hot plate) & anti-inflammatory activity was determined by three methods (carrageenan, formalin induced paw edema & cotton pellet granuloma) at doses 200 & 400mg/kg body weight in experimental animals using diclofenac sodium, tramadol, Indomethacin as reference drugs.

Results: In all the animals models the results obtained were statistically significant ($P<0.05$) in comparison to control.

Conclusion: In those animal models, the results obtained indicate that *Sterculia foetida* has significant analgesic and anti-inflammatory activities.

Keywords: *Sterculia foetida*, analgesic activity, anti-inflammatory activity.

INTRODUCTION

To a part of the body, pain is unpleasant sensation. It is both sensation and emotion. Pain occurs usually when peripheral nociceptors are stimulated in response to visceral distension, tissue injury or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system (Braunwald et al., 2008). Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as damaged cells, pathogens, or irritants. To remove the injurious stimuli as well as initiate the healing process for the tissue, it is a protective attempt by the organism. 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 (Anilkumar, 2010). 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 (Kaushik et al., 2012). 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.

Sterculia foetida belongs to the family Sterculiaceae. Extract of leaves on various animal models showed CNS depressant activity and antiinflammatory activity observed as decreased exploratory activity in mice and potentiation of pentobarbitone sleeping time in normal and chronic pentobarbitone-treated mice. Study yielded tannins, 2-deoxysugars, leucoanthocyanin and benzopyrone nucleus. Results showed extracts with antibacterial activity, inhibiting S aureus and *E. coli*. Therefore the present study was undertaken to investigate analgesic and anti-inflammatory activity of ethanolic and aqueous flower

extract of *Sterculia foetida* (Madhavachetty et al., 2008).

MATERIALS AND METHODS

Plant material

The flower of *Sterculia foetida* 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 flower was kept in the Museum of Sunrise University.

Preparation of ethanolic flower extract

The flower of *Sterculia foetida* 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. Under reduced pressure using a rotary evaporator, the extract was concentrated. The yield of the extract was found to be 13.5 %. Until further use, extract was preserved in a desiccator.

Preparation of aqueous flower extract

The flower of *Sterculia foetida* were shade dried and powdered. For 7days, the aqueous extract was prepared by cold maceration. The powder were soaked in distilled water and stirred intermittently and then left overnight. Macerated flower 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 12.75%.

Preliminary Phytochemical screening

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

Animals

Albino rats of Sprague Dawley strain (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 body weight (OECD, 2001).

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 ^{0}C. 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 *Sterculia foetida* (200, 400 mg/kg body weight), saline (vehicle) and standard drug (Tramadol 30 mg/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 (Singh et al., 1996).

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. *Sterculia foetida* ethanolic & aqueous extracts at dose (200mg/kg, 400mg/kg body weight), 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 *ad 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^{0}C.Animals having latency time greater than 15 seconds 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 (Kulkarni, 2007).

ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced paw edema in rats

Albino rats of Sprague Dawley strain weighing 170-200 gms of either sex were used. *Sterculia foetida* ethanolic & alcoholic extracts at dose (200 mg/kg, 400 mg/kg body weight), 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 (Winter et al., 1962) .

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 (Singh et al., 1996).

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 *Sterculia foetida* (200, 400 mg/kg body weight), Diclofenac (10 mg/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 8^{th} day. The granulation tissue with cotton pellet was dried at 60^0C 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 (Winter and Porter, 1957).

RESULTS

Acute toxicity studies

The flower extract of *Sterculia foetida* 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 2000 mg/kg body weight dose. Based on this test *Sterculia foetida* was tested at 200 & 400 mg/kg body weight for this experiment.

Phytochemical screening

The ethanolic & aqueous extract of *Sterculia foetida* 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 *Sterculia foetida* ethanolic and aqueous flower extract measured by tail immersion method are given in Table-1. At dose 200, 400 mg/kg *Sterculia foetida* 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.

Table 1. Analgesic activity of *Sterculia foetida* using tail immersion method in rats.

Groups	Reaction Time (Seconds)					
	Dose mg/kg body weight	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**
Sterculia foetida Ethanolic extract	200	2.40 ± 0.049*	3.48 ± 0.172*	3.86 ± 0.363*	3.70 ± 0.180*	3.56 ± 0.363*
Sterculia foetida Ethanolic extract	400	2.50 ± 0.223ns	3.92 ± 0.409ns	3.24± 0.163**	3.10 ± 0.100ns	3.24± 0.163**
Sterculia foetida Aqueous extract	200	2.39 ± 0.071ns	3.95 ± 0.057ns	3.98 ± 0.114**	3.66 ± 0.174*	3.28 ± 0.114**
Sterculia foetida Aqueous extract	400	2.38 ± 0.143*	3.88± 0.273**	3.76± 0.191*	3.50 ± 0.283*	3.26± 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

Hot plate method in rats

Results of analgesic activity of *Sterculia foetida* ethanolic and aqueous flower 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 200 mg/kg for aqueous extract and the percentage inhibition was found to be 51.95%, when compared to 400 mg/kg aqueous extract which was found to be 44.13%, whereas Tramadol showed 83.91% inhibition when compared to control.

Table 2. Analgesic activity of *Sterculia foetida* by hot plate method in rats.

Groups	Reaction Time (Seconds)					
	Dose mg/kg body weight	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**
Sterculia foetida Ethanolic extract	200	8.40 ± 0.049*	8.98 ± 0.172*	9.76 ± 0.363*	9.70 ± 0.180*	9.86 ± 0.363*
Sterculia foetida Ethanolic extract	400	8.50 ± 0.223ns	9.12 ± 0.409ns	10.24± 0.163**	11.10 ± 0.100ns	11.24± 0.163**
Sterculia foetida Aqueous extract	200	8.39 ± 0.071ns	9.25 ± 0.057ns	10.98 ± 0.114**	11.66 ± 0.174*	13.98 ± 0.114**
Sterculia foetida Aqueous extract	400	9.38 ± 0.143*	10.08± 0.273**	11.26± 0.191*	12.50 ± 0.283*	13.26± 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 *Sterculia foetida* on paw edema induced by Carrageenan in rats.

Groups	Dose mg/kg body weight	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**
Sterculia foetida Ethanolic extract	200	0.61± 0.055*	0.70± 0.120**	0.82 ± 0.111*	0.77± 0.129*	0. 88 ± 0.092ns	0.98 ± 0.123ns
Sterculia foetida Ethanolic extract	400	0.57± 0.120**	0.65 ± 0.111*	0.78± 0.129*	0.80 ± 0.146ns	0.88± 0.120*	0. 97± 0.055*
Sterculia foetida Aqueous extract	200	0.58 ± 0.065**	0.69 ± 0.070ns	0.73 ± 0.006*	0.86 ± 0.102ns	0.90 ± 0.084**	0.99 ± 0.119*
Sterculia foetida Aqueous extract	400	0.57 ± 0.050*	0.62 ± 0.100ns	0.75 ± 0.065*	0.64± 0.059*	0.87 ± 0.061**	0.97 ± 0.015ns

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.

Anti-inflammatory Activity

Carrageenan- Induced paw edema in rats

Results of anti-inflammatory activity of *Sterculia foetida* ethanolic and aqueous flower extracts are given in Table-3. Injection of Carrageenan was done 1h after oral administration of the extract (200,400 mg/kg body weight), Indomethacin (reference drug). Both the ethanolic and aqueous flower extracts showed significant inhibition of paw edema at 3h. Ethanolic extract showed 50.00%, whereas aqueous extract showed 60% at dose 400 mg/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 *Sterculia foetida* ethanolic and aqueous flower 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 2hr and significant up to 5th hr. Ethanolic extract showed 59.55% inhibition, whereas aqueous extract showed 63.97% 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 *Sterculia foetida* ethanolic and aqueous flower extract are given in Table-5. The flower 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 32.69%, 48.07%, 50.96%, 48.07% respectively. Diclofenac (reference drug)

inhibited granuloma tissue formation with a value of 93.31%.

Table 4. Anti-inflammatory activity of *Sterculia foetida* on paw edema induced by formalin in rats.

Groups	Dose mg/kg body weight	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
Sterculia foetida Ethanolic extract	200	0. 55±0.015^{ns}	0.69 ±0.124^{ns}	0.55 ± 0.102^{ns}	0.76±0. 092**	0.82± 0.091*	0.86± 0.094*
Sterculia foetida Ethanolic extract	400	0.56 ± 0.007*	0.59 ±0.042*	0. 52± 0.024*	0.83±0.023*	0.83 ± 0.144*	0.87±0 .052*
Sterculia foetida Aqueous extract	200	0. 60 ±0.049^{ns}	0.66± 0.047^{ns}	0.56 ± 0.035*	0.88± 0.700*	0.86± 0.632*	0.96±0 .118*
Sterculia foetida Aqueous extract	400	0.59 ± 0.082^{ns}	0.60 ± 0.118^{ns}	0. 49± 0.044*	0.87± 0.128*	0.85± 0.079**	0.92±0 .122*

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 *Sterculia foetida* 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**
Sterculia foetida Ethanolic extract	200	140±4.964 ns
Sterculia foetida Ethanolic extract	400	108±4.589**
Sterculia foetida Aqueous extract	200	102±3.071 ns
Sterculia foetida Aqueous extract	400	108±2.836**

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.

DISCUSSION

In the present study analgesic activity of *Sterculia foetida* ethanolic and aqueous flower 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. Standard reference drugs used are Diclofenac sodium, Tramadol &, Indomethacin. 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 only peripheral pain was inhibited by non-steroidal anti-inflammatory drugs (Elisabetsky, 1995).

The analgesic effect of *Sterculia foetida* ethanolic and aqueous flower 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 (Gupta et al., 2006). 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 (Swingle and Shideman, 1972), 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 *Sterculia foetida* ethanolic and aqueous flower 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.

REFERENCES

Anilkumar M. Ethnomedicinal plants as anti-inflammatory and analgesic agents, in Ethnomedicine: A Source of Complementary Therapeutics. Research Signpost, Kerala, India, 2010.

Braunwald E, Fauci AS, Fields HL, Kasper DL, Hauser SL, Longo DL, et al. Pain pathophysiology and management In: Harrison's Principles of internal medicine (17th edition). New York; McGraw Hill: 2008.

Elisabetsky E, Amador TA, Albuquerque RR, Nunes DS, Carvalho Ado C. Analgesic activity of *Psychotria colorata* (Willd. ex R. & S.) Muell. Arg. alkaloids. Journal of Ethanopharmacology. 1995; 48(2):77-83.

Gupta M, Mazumder UK, Gomathi P, Thamil Selvan V. Anti-inflammatory evaluation of leaves of *Plumeria accuminata*. BMC complementary and Alternative Medicine. 2006;6:1-6.

Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and anti-inflammatory activity of *Pinus roxburghii Sarg.* Advances in Pharmacological Sciences. 2012;(2012) article ID 245431; 1-6.

Kulkarni SK. Handbook of experimental pharmacology 9th edition. Vallabah Prakashan, New Delhi, India: 2007.

Madhavachetty K, Sivaji K, Rao KT. Flowering plants of Chittoor district Andhra Pradesh, India. 2nd edition. Students Offset Printers, Tirupati, India.

OECD. OECD/OCDE. OECD (423) guidelines for testing of chemicals. Acute oral toxicity up and down procedure, 2001:1-26.

Singh S, Majumdar DK, Rehan HM. Evaluation of anti-inflammatory potential of fixed oil of *Ocimum sanctum* (Holybasil) and its possible mechanism of action. Journal of Ethnopharmcology. 1996; 54(1):19-26.

Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. Journal of Pharmacology and Experimental Therapeutics. 1972;183(1):226-234.

Winter CA, Porter CC. Effect of alterations in the side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. Journal of the American Pharmaceutical Association. 1957; 46:515-519.

Winter CA, Risley EA, Nus GW. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine. 1962;111:544-547.