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Evaluation of Anti-Inflammatory Activity of Methanolic Extract of Fruits of *Solanum Xanthocarpum* Schrad & Wendl in Albino Rats

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Abstract—
Objective*: To evaluate the anti-inflammatory activity of methanolic extract of Solanum Xanthocarpum in albino rats.*
Materials and Methods*: Methanolic extract in the dose of 100,200,400mg/kg and aspirin in the dose of 100mg/kg was administered orally in different groups of albino rats to study their effect on inflammation induced by carrageenan or a foreign body. Aspirin100mg/kg and extract 400mg/kg was administered to study their interaction.*
Results: *Methanolic extract of fruits of solanum xanthocarpum produced significant anti-inflammatory activity in acute and subacute inflammation models and was comparable to that of aspirin. The extract also potentiated the anti-inflammatory action of aspirin.*
Conclusion*: Methanolic extract of fruits of solanum xanthocarpum possess significant anti-inflammatory activity.*

Keywords— *Anti-inflammatory; methanolic extract; solanum xanthocarpum*.

I. INTRODUCTION

Plants have been the basis of many traditional medicine systems throughout the World for thousands of years and still remain as the main new source of structurally important chemical substances that lead to the development of innovative drugs [1]. The use of medicinal plants for the treatment of many diseases is associated with folk medicine from different parts of the World [2]. Our North-East India including Tripura is very rich in plants and herbs because of plenty of rainfall and availability of deep forest.

Inflammation is a great area of interest for research for many years. Inflammation is a complex reaction to injurious agents of vascularized living tissue. It is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. Acute inflammation is a rapid response to an injurious agent that serves to deliver mediators of host defense like leucocytes & plasma proteins to the site of injury. The chronic inflammation is of prolonged duration in which active inflammation, tissue destruction and attempts at repair are proceeding simultaneously. Edema formation, leukocyte infiltration & granuloma formation represents components of inflammation [3].

Drugs which are in present use for the management of pain & inflammatory conditions are Opioid, Non-Steroidalanti-inflammatory drugs (NSAIDs) and corticosteroids. Although these drugs are effective in controlling signs of inflammation, numbers of adverse effects encountered are the biggest limitations to their use.

There is continuous search for safer alternate anti-inflammatory agents. Many indigenous preparations have shown good promise in controlling inflammation. A survey of Ayurvedic literature reveals that the anti-inflammatory activity of Solanum genus, belonging to Solanaceae family has been successfully used in Ayurvedic and other traditional formulations and they are found to be efficacious and cheap as compared with modern conventional drugs [4].

Solanum xanthocarpum (Sx) isan herb that grows in Uttar Pradesh, Bihar, Uttaranchal, Punjab, West Bengal & North Eastern states of India. It widely grows in hills & valleys of Tripura. Fruits are edible & local tribal people use fruits for treatment of various ailments as traditional folk medicines. Juice of fruit of Sx is used in sore throats & rheumatism. The hot aqueous extract of dried fruits is used to treat cough, fever. The fruit paste is applied externally to the affected area for treating pimples and swellings [5]. The fruits are reported to contain several steroidal alkaloids like Solanacarpine & Solamargin. Other constituents like Carpesterol, Stigmasterol, Diosgenin shows anti-inflammatory effect [6]. The present work is taken up to evaluate the anti-inflammatory analgesic, antipyretic activity of methanolic extract of fruits of solanum xanthocarpum.

II. MATERIALS AND METHODS

Animals:

Male albino Wistar rats weighing 150-200 gm were used for the study. They were housed in standard polypropylene cages under room temperature and exposed to 12:12 hr. light: dark cycle. The rats were fed with standard diet and water ad libitum. Institutional animal ethics committee approved the study.

Procurement & Authentication of Plant Samples:

Whole plants of *Solanum Xanthocarpum* was collected from college campus (Tripura Medical College & DR Bram

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Teaching Hospital, Hapania) from the month of August - September 2014 & authenticated by botanist from Tripura University.

Preparation of Plant Material

The shade dried powder of fruits was extracted in a soxhlet apparatus with methanol. The yield at the end of extraction was 30%. The extract was suspended in 1% gum acacia with distilled water and a concentration of 50mg/ml was prepared.

Phytochemical Studies

The Methanolic extract was subjected to qualitative chemical tests for the detection of phytoconstituents according to the methods of Seikel MK (1964) [7] and Kokate CK (1994) [8]. The preliminary tests showed presence of flavonoid compounds.

Toxicity Study

The acute toxicity testing was done as per OECD guidelines 423 on albino rats. [9] Five animals were used. Three doses -100 mg, 200mg and 400mg were selected for the study.

Carrageenan Induced Paw Edema

The anti -inflammatory activity of Methanolic extract of *Solanum Xanthocarpum* was evaluated by the method described by Winter CA *et al.* (1962) with slight modifications. [10] Rats were divided into six groups of six animals each. They were starved overnight with water ad libitum prior to the day of experiment. The control group received 0.5ml of 1% gum acacia suspension orally. The other groups received the methanolic extract in the dose of 100, 200,400mg/kg, aspirin 100mg/kg and another group received the extract in 400mg/kg dose as well as aspirin 100mg/kg. Acute inflammation was induced by injecting 0.1 ml of 1% Carrageenan in normal saline into the sub plantar region of the right hind paw, thirty minutes after oral administration of drugs. A mark was applied on the leg at the malleolus to facilitate subsequent readings. The paw edema volume was measured by mercury displacement with the help of a plethysmograph at 0hr, 0.5hr, 1hr, 3hr, and 5hr after injecting Carrageenan. The difference between 0hr and subsequent readings was considered as oedemavolume. The percentage inhibition of oedema in various groups was calculated using the formula:

$$\text{Percent oedema inhibition} = [1\text{-}V_t/V_c] \text{ X } 100$$

Vt and Vc were mean oedema volume in the drug treated and control groups respectively. [11]

Carrageenan Induced Air Pouch Model

The rats were divided into six groups (n=6). Air pouch was produced according to the method of described by Salvemini et al. Rats were anesthetized and air cavities were produced by subcutaneous injection of 20 ml of sterile air into the intracapsular area of the back. On the 3rd and 6th day an additional 10ml of air was injected into the cavity to keep the space open. On the 7th day 2ml of 1% solution of carrageenan dissolved in saline was injected directly into the pouch to induce an inflammatory response. The six groups of rats received similar treatment as above. The second dose of treatment was repeated after 24hrs of the first treatment. After 48 hrs of carrageenan injection the rats were anesthetized with ether and the pouch was carefully opened by a small incision. The volume of exudates was collected and measured. An aliquot of the exudates was used for quantification of leucocyte concentration using a hemocytometer and differential cell count was performed using a manual cell counter after staining with Wrights stain. The results were expressed as the total number of neutrophils and monocytes.

Cotton Pellet Induced Granuloma

Sub-acute inflammation was induced by slightly modified method of D Arcy *et al.* [12]

Rats were divided into six groups (n=6). After 1 hour of drug administration under light ether anaesthesia two sterile cotton pellets weighing 10 mg each was implanted in the back through a small subcutaneous incision according to the method described by Swingle and Shideman [13]. The wounds were then sutured and the animals were caged individually after recovery from anesthesia. Aseptic precautions were taken throughout the experiment. All the drugs were administered daily at 24 hrs. interval for 10 days.

On the 11th day the rats were anaethetised with ether. The cotton pellets were removed, weighed, and dried in a hot air oven at 60^0C for 18 hrs. And their dry weight was measured. Net Granuloma formation was calculated by subtracting the initial weights of cotton pellets from the final weights .Mean granuloma wet weight, dry weight, transudative weight and granuloma weight for the various groups was calculated. Body weight was measured in all the animals. The level of inhibition of granuloma tissue development was calculated using the formula:

$$(Tc - Tt/Tc) \times 100$$

Where Tc = Weight of granuloma tissue of the control group

Tt = Weight of granuloma tissue of treated group [14]

Statistical Analysis: Data were expressed as mean ± SEM and were analyzed by the one way ANOVA followed by the Dunnet′s test. $P < 0.05$ was considered significant.

III. RESULTS

Effect of Methanolic extract of fruits of *Solanum Xanthocarpum* on Carrageenan induced paw oedema in rats (paw volume in ml)

Treatment Groups (n=6)	Dose	0 hr.	0.5 hr.	1 hr.	3hr.	5 hr.
Control	0.5ml1%gum acacia	0.95 ± 0.002	0.98 ± 0.003	0.98 ± 0.003	1.01 ± 0.02	1.00 ± 0.01
Methanolic extract	100mg/kg	0.89 ± 0.01a	0.86± 0.005a	0.84 ± 0.008	0.83 ± 0.02a	0.78 ± 0.03a
Methanolic extract	200mg/kg	0.92±0.008b	0.90 ± 0.01a	0.88 ± 0.008a	0.85 ± 0.003a	0.82±0.003a
Methanolic extract	400mg/kg	0.80±0.008a	0.79± 0.003a	0.77 ± 0.006a	0.75 ± 0.01a	0.73±0.003a
Aspirin	100mg/kg	0.92±0.003b	0.91± 0.003a	0.89 ± 0.003a	0.86 ± 0.05a	0.82±0.003a
ME + Aspirin	(400 + 100) mg/kg	0.97±0.003b	0.99± 0.003a	0.94 ± 0.003a	0.89 ± 0.05a	0.88±0.003a

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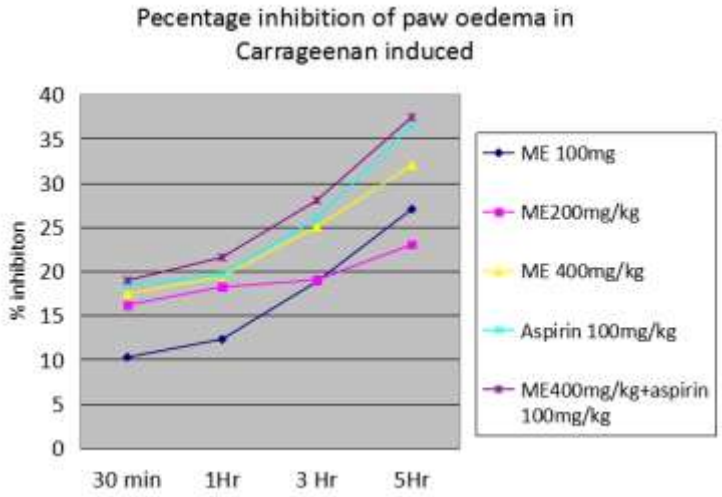
Each value is mean ± SEM N=6 rats
a P < 0.01
b P < 0.05
One way ANOVA followed by Dunnett's t test
Statistically significant when compared to control

Percentage inhibition (%) at various times interval					
Treatment	**0.5 hr.**	**1 hr.**	**3 hr.**	**5 hr.**	**Mean of % inhibition**
ME (100mg/kg)	10.24	12.28	18.82	27.00	17.08
ME (200mg/kg)	16.16	18.20	19.00	23.00	19.09
ME (400 mg/kg)	17.38	19.42	25.00	32.00	23.45
Aspirin (100 mg/kg)	18.43	19.55	26.01	36.42	25.10
ME + Aspirin (400 mg/kg +100 mg/kg)	18.93	21.55	28.01	37.42	26.10

Effect of methanolic extract of solanum xanthocarpum on leucocyte infiltration & exudates volume in carrageenan induced air pouch model

DRUG	DOSE (MG/KG) BW	EXUDATES VOLUME	NEUTROPHILS (X 10^6 CELLS)	MONOCYTES (X 10^6 CELLS)
Control	0.5ml 1% gum acacia	3.76 ± 0.04	286. 2 ± 2.34	124.04 ± 1.01
ME	100	0.94 ± 0.03	102.0 ± 1.41	62.17 ± 1.13
ME	200	0.99 ± 0.22	104.0 ± 1.13	63.12* ± 1.03
ME	400	0.81* ± 0.01	100.2** ± 1.25	68.24 ** ± 1.15
Aspirin	100	0.59** ± 0.33	95. 4** ± 1.05	65.02*** ± 1.05
ME + Aspirin	400 + 100	0.48** ± 0. 05	89.2*** ± 1.15	63.04***± 1.05

Data analyzed by ANOVA followed by Dunnet′ s test. ***p < 0.001 **p< .01 *p<.05 when compared to control



Cotton Pellet Induced Granuloma

Effect of methanolic extract of fruits of *Solanum Xanthocarpum* on Cotton pellet induced granuloma in albino rats.

Group	Dose	Granuloma wet weight (mg)	% inhibition	Granuloma dry weight (mg)	% inhibition	Transudative weight (mg)	Granuloma weight (mg)	% of body weight gain
Control	0.5ml1%gum acacia	41.07±2.62		31.10±1.01		31.07±2.62	9.97±1.61	6.76
Aspirin	100mg/kg	16.84±2.47*	4.06	10.21±2.32*	8.78	6.84±2.47*	6.63±0.15*	7.09
ME	100mg/kg	37.73±2.40	3.46	26.43±1.01	5.43	27.73±2.40	11.3±1.40	6.56
ME	200mg/kg	23.31±2.03*	3.75	15.13±1.15*	5.86	13.31±2.03*	8.18±0.88*	6.61
ME	400mg/kg	20.51±4.40*	3.84	12.32±2.41*	6.01	10.51±4.40**	8.19±1.99	6.66
ME+ASPirin	400mg+100mg	11.53±4.17*	4.17	5.63±3.43**	8.86	1.53±4.17***	5.9±0.74**	6.82

Each value is mean ± SEM N=6 rats
**P < 0.01, *P < 0.05, ***P < 0.001
One way ANOVA followed by Dunnet test

The results were statistically significant when compared to control

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IV. DISCUSSION

The most widely used primary test to screen new anti-inflammatory agents' measure the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent (Winter *et al*., 1962). Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito and Antonio, 1998; Gupta *et al*., 2006) [15], [16] The significant inhibitory activity shown by the extract of *Solanam Xanthocarpum* berries over a period of 5 h in carrageenan induced inflammation was quite similar to that exhibited by the group treated with aspirin .Previous study with some other plants like *Solanum trilobatum* (Pandurangan *et al*. 2008; 2009) [17], *Plumeria acuminate* (Gupta *et al*. 2006) [16]and *Thesiumchinense* (Parveen *et al.* 2007) [18] also showed the same effect in this model. These results indicate that the extract acts in later phases in dose dependent manner, probably involving arachidonic acid metabolites, which produce an oedema dependent on neutrophils mobilization (Just *et al*. 1998). [19] This effect is similar to that produced by non-steroidal anti-inflammatory drugs such as aspirin whose mechanism of action is inhibition of the cyclo oxygenase enzyme.

To assess the efficacy of the methanolic extract of solanum xanthocarpum against proliferative phases of inflammation we selected carrageenan induced air -pouch model in which tissue degradation and fibrosis occurs. During the repair process of inflammation there is proliferation of macrophages, neutrophils fibroblasts and multiplication of small blood vessels occurs which are the basic sources of forming a highly vascularized reddish mass, termed granulation tissue. Thus, in this model the methanolic extract significantly reduced infiltration of neutrophils and monocytes. The results indicate that methanolic extract of solanum xanthocarpum may alter the action of endogenous factors that are involved in migration of these substances to the site of inflammation. [20] Their is increased evidence that lysosomal enzymes play important role in the development of acute and chronic inflammation. Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosomal membrane which is a major event responsible for the inflammatory process. [21] Therefore it can be assumed that our drug extract might be acting by either inhibiting the lysosomal enzymes or stabilizing the membranes.

The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation and is a typical feature of established chronic inflammatory reaction. The moist /wet weight of the cotton pellets correlates with the transudate and the dry weight correlates well with the amount of granulomatous tissue formed. In chronic inflammation, monocyte infiltration and fibroblast proliferation take place. There is widespread proliferation of small vessels or granuloma. NSAIDs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides. [22] In the present investigation, the methanolic extract of *Solanum Xanthocarpum* showed a significant decrease in the wet weight and dry weight, transudative weight and granuloma weight of the cotton pellet granuloma indicating that chronic administration of the drug reduces the proliferation of fibroblast and synthesis of collagen and mucopolysaccharides as well as fluid accumulation in chronic inflammation.

The fruits of Solanum xanthocarpumschrad and wendl contain flavonoids, alkaloids, tannins and sterols. Various flavonoids, glycosides and aglycones were previously reported as having potent anti-inflammatory activity. Pourmotabbed et al., suggested that some flavonoids block both cyclooxygenase and lipooxygenase pathway of the arachidonate cascade at high concentration, while at low concentration only lipooxygenase pathway is blocked. [23] Also there are few reports on the role of tannins in anti-inflammatory activity. [24] In the present study the antiinflammatory activity of Solanum Xanthocarpum might be due to presence of flavonoids, sterols, saponins and tannins.

V. CONCLUSION

From the present study we come to the conclusion that the methanolic extract of fruits of *Solanum Xanthocarpum* has anti-inflammatory, properties in experimental animal models. Presence of flavonoid compounds and sterols and saponins may attribute to its above mentioned pharmacological activities. The present study also substantiates the traditional use of *Solanum Xanthocarpum* fruits for the treatment of various inflammatory ailments and suggests the presence of biologically active components which may be worth further investigation and elucidation.

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