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Anti-Inflammatory Activity of Cow Urine Betel Vine Extract in Rats

N.B. Sridharamurthy, M.N. Manasa ,B.A. Sowmya

***Abstract*— Piper betel (Betel vine) is a Vedic plant, which is using as a remedy for various diseases. In Ayurveda betel leaf extract was frequently used as an adjuvant and mixed with different medicines possibly for better effects beside its independent use as medicine. In addition to this, the aphrodisiac effect of it has been indicated in ancient texts. It is also believed to provide strength to the heart and regulate blood flow to the heart. Its utility as anti-inflammatory and anti-microbial is emphasized in several ancient books. According to Ayurveda it also helps in expelling the mucus from the respiratory tract. In Unani system of medicine it is described to improve taste and apetite, tonic to brain, heart and liver, lessens thirst, clears throat and purifies blood. Cow urine is believed to have therapeutic value and used in many drug formulations. Essentially, cow urine is used as disinfectant and for purification. With an approximate shelf life of around 5 years, this has proved to be the most effective natural antiseptic and disinfectant, when compared to the synthetic chemicals which are currently available to the consumers. Thus, it strengthens the fact that cow's urine is not a toxic effluent as 95% of its content being water, 2.5% urea and the remaining 2.5%, a mixture of minerals, salts, hormones and enzymes. In the rural villages in India, cow's urine is being used since a very long time as an effective antiseptic for wounds, skin diseases, bathing, etc. Fresh leaves of the Piper betel (Betel vine) were collected from the nearest plantation and leaves were authenticated, shade dried and powdered to get moderate coarse powder. The dry powder was then extracted with cow urine at 40-450C by maceration process for 72 hrs. Later the cow urine extract was filtered and the filtrate was concentrated to a semi solid mass by using vacuum distillation apparatus. The extract so obtained was used for the phytochemical and pharmacological investigations. In the present study, oral administration of the Cow urine Betel vine extract at the doses of 250mg/kg and 500mg/kg significantly inhibits paw edema and the results were compared with Standard Diclofenac (5mg/kg) treated group.**

***Index Terms*— Piper betel, Diclofenac, Cow urine.**

I. INTRODUCTION

Herbal Medicine is the oldest form of medicine known to mankind. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in India, China, and Egypt, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rig-Veda, Athervan veda, Charak samhita and Sushruta Samhita. It was the mainstay of many early civilizations and still the most widely practiced form of medicine in the world today (according to World Health Organization figures) (Kamboj V.P, 2000).

Great interest in herbal medicine as a potential source of phytopharmaceuticals has created the need to review common factors responsible for major diseases and body disorders. This review shows one such common factor in inflammation and the role herbal medicine can play. Traditional herbal remedies have long been used to treat various pain or inflammation related diseases and various pro-inflammatory mediators in diseases and disorders. These mediators have therefore been suspected of being the functional basis of diseases and disorders. The structural diversity of these medicinal herbs makes them a valuable source of novel lead compounds against the therapeutic molecular targets, cytokines and mediators that have been newly discovered by the platforms of genomics, proteomics and metabolomics and high throughput technologies (Iwalewa E.O, 2007).

II. MATERIALS & METHODS

A. Preparation of extract of test drug

Fresh leaves of the of Piper betel (Betel vine) were collected from the nearest plantations. The plant was authenticated by Dr. Muralidhar, Professor and HOD, Department of Biotechnology, Department of Life Science, Dayananda Sagar Institutions, Banagalore-78. The leaves were cut down into small pieces, shade dried and powdered to get moderately coarse powder, which was sieved under suitable mesh. About 500gms of dry powder was extracted with cow urine at 40-45 degree Celsius by maceration process for a period of 72 hrs. Later the cow urine extract was filtered and concentrated to a semi solid mass by using vacuum distillation apparatus. The extract so obtained was dark brown in color.

Acute toxicity studies:

LD50 of cow urine Piper betel extract was determined as per OECD guidelines-425.

Healthy Wister rats of either sex weighing between 150 to 200 gms were employed but the females should be non pregnant. The test substance was administered in a single dose by gavages. Six animals were used for each step. The maximum dose selected was 5000mg/ kg b.w. After dosing the animals were kept for observation for period of 72 hrs for any physical change and mortality. During the time animals were allowed for free accesses to water. [95].

Experimental study

Evaluation of anti-inflammatory activity of the Cow urine Betel vine extract:

N.B.Sridharamurthy, Department of Pharmacology, School of Pharmaceutical Sciences
M.N. Manasa, Department of Pharmacology, School of Pharmaceutical Sciences
B.A. Sowmya, Dayananda Sagar University, Kumar swamy layout

Carrageenan induced paw edema(Winter, 1962):

a. Preparation of test samples:

Suspension of crude extract of Piper betel was prepared by using cow urine as solvent so as to obtain the dosage of 250mg/kg and 500mg/kg body weight. These suspensions were administered orally to the animals.

b**. Preparation of standard drug formulation:**

Diclofenac was suspended in water and administered orally to the animals at the dose of 5mg/kg body weight.

c. Procedure:

Group 1- Negative Control (normal saline)

Group 2- Standard treated group Diclofenac (5mg/kg, p.o).

Group 3- Positive Control (Cow urine)

Group4- Lower dose of the extract (250 mg/kg, p.o)

Group 5- Higher dose of the extract (500 mg/kg, p.o)

Wistar rats of either sex weighing between 150-200g were used for the study and fasted overnight prior and during the experiment but have allowed free access to water. The rats were divided into 5 groups of six animals each. A mark was made on the hind paw just below the tibio-tarsal junction so that every time the paw could be dipped in the colored solution of the column of the plethysmograph to ensure constant paw volume. After 1 hour of above treatments, inflammation was induced by injecting 0.1ml of 1% w/v of carrageenan in 0.9% sodium chloride into plantar surface of the right hind paw of the animals. Paw volumes were measured using a Plethysmograph at different time intervals of 0, 30, 60,120 and 240 minutes. The reduction in the paw volume was calculated. The percentage inhibition of edema can be calculated using the following formula.

% Inhibition of Edema= [1-(Vt/Vc)] x 100

Where Vt is edema volume of the drug treated group and Vc is the edema volume of the control group.

Histamine induced paw edema (Saleh 1999):

Group 1- Negative control (normal saline)

Group 2- Standard group Diclofenac at the dose (5mg/kg, p.o).

Group 3- Positive control Cow urine (1 ml)

Group 4- Lower dose of Cow urine Betel vine extract (250 mg/kg, p.o)

Group 5- Higher dose of Cow urine Betel vine extract (500 mg/kg, p.o)

Wistar rats of either sex weighing between 150-200g were used for the study. The animals were fasted overnight prior and during the experiment but have allowed for free access to water. The rats were divided into 5 groups of six animals each. A mark was made on the hind paw just below the tibio-tarsal junction so that every time the paw could be dipped in the colored solution of the wider column of the plethysmograph up to the mark to ensure constant paw volume. After 1 hour of above treatment, the inflammation was induced by injecting 0.1ml of 1% of histamine into sub-plantar surface of the right hind paw of the animals. Paw volumes were measured using a Plethysmograph at different time intervals of 0, 30, 60, 120 and 240 minutes. The reduction in the paw volume was calculated. The percentage inhibition of edema can be calculated using the following formula.

% Inhibition of Edema= [1-(Vt/Vc)] x 100

Where Vt is the edema volume of the drug treated group and Vc is the edema volume of the control group.

RESULTS

Anti-inflammatory activity of Cow urine Betel vine extract

Carrageenan induced paw edema

The animals were pretreated with extract at the doses of 250mg/kg, 500mg/kg and the standard Diclofenac 5mg/kg b.w. respectively. Among the selected doses extract II (500mg/kg) showed a reduction in the paw volume induced by carrageenan which is 0.74±0.03 when compared to the control 0.9±0.04. Extract I (250mg/kg) showed a little reduction in the paw volume of 0.80±0.04.The results are tabulated in Table No.1 and Fig No.1.The results were significant at P<0.05.

All the values are expressed as Mean±SEM (n=6) by one way ANOVA followed by Dunnet test *P<0.05, **P<0.01 when compared to control group.

KEY: **NST**: Normal saline treated; **STD: Standard** diclofenac**; PC: Positive** control-cow urine;**TE I:**Test extract I;**TE II:**Test extract II

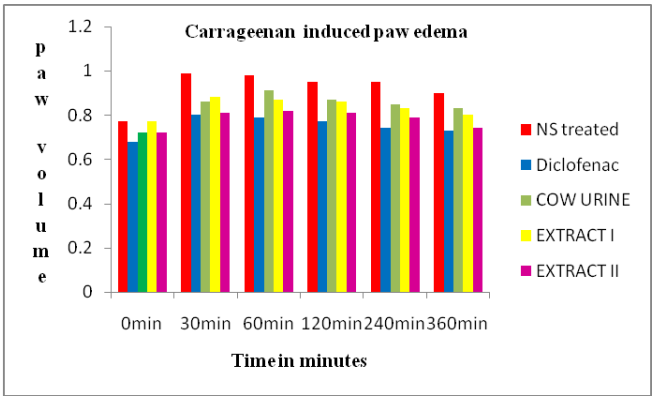

Fig No.1: Anti-inflammatory activity of Cow urine Betel vine extract on Carrageenan induced paw edema.

Histamine induced paw edema

The animals were pretreated with the extract at the doses of 250mg/kg, 500mg/kg b.w. respectively, and with and Diclofenac 5mg/kg b.w taken as standard. Among the two doses extract II (500mg/kg) showed maximum reduction in the paw volume induced by Histamine which is 0.68±0.03 as compared to control 0.62±0.02. Extract I (250mg/kg) showed little reduction in the paw volume of 0.77±10.04 as compared to control. Results are tabulated in Table No.2 and Fig No.2 and are significant at P<0.05.

Table No.1: Anti-inflammatory activity of Cow urine Betel vine extract on Carrageenan induced paw edema.

		Difference in paw edema volume (Mean ±SEM)					
Groups	**Dose oral/kg**	**0 Min**	**30 Min**	**60 Min**	**120 Min**	**240 Min**	**360 Min**
NST	10ml	0.77± 0.05	0.99± 0.03	0.98± 0.03	0.95± 0.03	0.95± 0.01	0.9± 0.04
STD(Diclo)	5mg	0.68± 0.03	0.80± 0.04*	0.79± 0.03**	0.77± 0.03*	0.74± 0.02*	0.73± 0.02**
PC(urine)	1ml	0.72± 0.07	0.86± 0.04	0.91± 0.04	0.87± 0.03*	0.85± 0.04*	0.83± 0.02
TE I	250mg/	0.77± 0.04	0.88± 0.05*	0.87± 0.04*	0.86± 0.03	0.83± 0.04*	0.80± 0.04*
TE II	500mg	0.72± 0.05	0.81± 0.08*	0.82± 0.01*	0.81± 0.06*	0.79± 0.01*	0.74± 0.03**

Table No.2: Anti-inflammatory activity of Cow urine Betel vine extract on Histamine induced paw edema.

		Difference in paw edema Volume Mean±SEM					
Groups	**Dose oral**	**0 Min**	**30 Min**	**60 Min**	**120 Min**	**240 Min**	**360 Min**
NST	10ml/kg	0.47± 0.03	0.64± 0.03	0.922± 0.02	1.04± 0.01	0.87± 0.03*	0.85± 0.04**
Std (Diclo)	5 mg/kg	0.33± 0.02	0.44± 0.03*	0.58± 0.02*	0.66± 0.02	0.62± 0.02*	0.61± 0.03*
PC (Urine)	1ml	0.44± 0.03	0.55± 0.02*	0.80± 0.01*	0.91± 0.01*	0.86± 0.03	0.81± 0.06*
T E I	250mg/kg	0.41± 0.01	0.53± 0.03*	0.68± 0.01*	0.86± 0.01*	0.85± 0.04*	0.77± 0.04
TE II	500mg/kg	0.38± 0.07	0.48± 0.01*	0.62± 0.02*	0.77± 10.04	0.74± 0.04	0.68± 0.03

All the values are expressed as Mean±SEM (n=6) by one way ANOVA followed by Dunnet test *P<0.05, **P<0.01 when compared to control group.

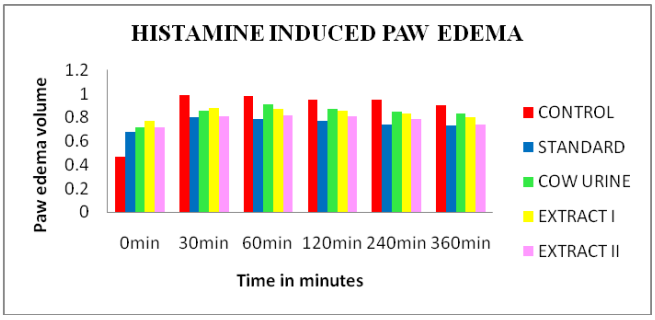

Fig No.2: Anti-inflammatory activity of Cow urine Betel vine extract on Histamine induced paw edema.

Fig No.3: Plethysmograph

III.DISCUSSION

Carrageenan-induced rat paw edema is a widely used test to determine the anti-inflammatory activity, and it has been fully characterized in the past (Spector, 1963). The development of carrageenan induced inflammatory reaction in rats' results from the activation of kinin system, the accumulation of leukocytes and release of several inflammatory mediators such as PGs and cytokines (Rosa M, 1972). In the present study, oral administration of the Cow urine Betel vine extract at the doses of 250mg/kg and 500mg/kg significantly inhibited paw edema volume as compared to Standard Diclofenac (5mg/kg) treated group.

The significant inhibition of paw edema volume in pretreated Cow urine Betel vine extract at the doses of 250mg/kg and 500mg/kg b.w was compared with Diclofenac treated animals in two selected models-Carrageenan induced and Histamine induced paw edema. The extract treated animals showed a significant decrease in paw volume edema as compared to negative control and standard treated group.

Table No.1 shows the results of Carrageenan induced paw edema model. Paw edema volume at 360th min of Test I and Test II treated animal are 0.80±0.04 and 0.74±0.03 respectively, and the paw volume of the control treated animals is 0.9±0.04. The paw edema volume of Standard treated group is 0.73±0.02 and that of cow urine treated group was 0.83±0.02. The values of the Test II and Standard results are similar; hence the Test drugs played a significant role in reducing the inflammation induced by carageenan.

Table No.2 shows the results of Histamine induced paw edema model. Paw volumes of Test I and Test II treated animal at 360th min are 0.77±0.04 and 0.68±0.03 respectively, and the paw volume of control treated animals is 0.85±0.04. The paw volume of the Standard treated group is 0.61±0.03.The value of cow urine treated group was found to be 0.81±0.06.This also shows that values of the Test extract II is equal to that of the standard in reducing the paw volume; hence the Test extract II significantly reduced the inflammation induced by Histamine.

CONCLUSION

The extract showed to possess significant anti-inflammatory activity. The phytochemical constituents like phenolic compounds and flavonoids may inhibit the inflammatory pathway and may inhibit the formation of cytokines and prostaglandins responsible for the inflammation. The anti-inflammatory potency was demonstrated in both the models selected for the investigation. The presence of phenolic compounds, tannins and flavanoids in the extract was further confirmed by phtyochemical screening. Thus the studies carried out provide a supportive scientific evidence for the medicinal use of Cow urine betel vine extract against inflammation, thereby justifying the use of the same in Indian traditional system of medicine.

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