Int. J. Chem. Sci.: 10(1), 2012, 548-556
ISSN 0972-768X
www.sadgurupublications.com

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME SELECTED SPEICES OF ASCLEPIADACEAE FAMILY

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ABSTRACT

The main objective of the present investigation is to evaluate the anti-inflammatory property of *Calotropis procera*, *Gymnema sylvestre* and *Hemidesmus indicus* in Wister rats using the Carrageenan induced left hind paw edema. In the study it was observed that the aqueous, ethanol and chloroform extracts were significantly inhibit paw edema induced by carrageenan in rats. The ethanolic extract showed a significant anti-inflammatory property when compared with the aqueous, chloroform, standard and untreated control. Among the three tested plant species *H. indicus* showed the maximum inhibition in rats.

Key words: Anti-inflammatory, Asclepiadceae family.

INTRODUCTION

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. Inflammatory responses occur in three distinct temporal phases, each apparently mediated by different mechanisms: 1) an acute phase characterized by transient local vasodilation and increased capillary permeability; 2) a delayed, sub-acute phase characterized by infiltration of leukocytes and phagocyte cells; and 3) a chronic proliferative phase, in which tissue degeneration and fibrosis occur (Katzung, 2001; Satoskar and Bhandarkar, 1995; Smith and

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Reynard, 1995; Udeme et al., 2009; Pramod et al., 2010). Inflammation is a defense mechanism, the complex events and mediators involved in the inflammatory reactions induce, maintain or aggravate many diseases (Sosa et al., 2002; Gupta et al., 2003; Ravikumar et al., 2006; Patil et al., 2009; Alam et al., 2011). There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls etc. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system. A lot of references are available in the field of ethnomedicinal plants used as anti-inflammatory drugs. Bagul et al. (2005) have reported the anti-inflammatory activity of two ayurvedic formulations containing 'guggul'. Bhattacharya et al. (2005) have reported anti-inflammatory potential of methanol extract of *Stepenia glabra* of Menispermaceae family. The extract depicted anti–inflammatory activity at the dose of 150 mg/kg body weight. Ammar et al. (1997) have revealed the anti-inflammatory activity of bioactive fractions isolated from seeds of *Trigonella foenum gracium* L., roots of *Glycyrrhiza glabra* L. and fruits of *Coriandrum sativum* L.

However, studies have been continuing on inflammatory diseases indicated that the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use (Kayaalp, 1998; Mahesh et al., 2009; Sudipta Das et al., 2010; Rajamanickam et al., 2010). Low cost and easy availability are the factors that generated a renewed interest in plant medicine in the last decade. The traditional practitioners in India prescribe the leaves to the patients without regard to any possible adverse effects in the view of its many uses. Therefore, development of newer, more powerful anti-inflammatory drugs lesser side effects are necessary. Hence the present study planned to evaluate the effect of aqueous, methanol and chloroform extracts of the leaves of *Calotropis procera*, *Gymnema sylvestre* and *Hemidesmus indicus* on anti-inflammation in rats.

EXPERIMENTAL

For the evaluation, the leaves of *Calotropis procera*, *Gymnema sylvestre* and *Hemidesmus indicus* were collected from the Campus of Acharya Nagarjuna University Area Guntur District Andhra Pradesh in the month of July 2011. After cleaning the leaves were shade dried at room temperature for 10 days and coarsely powdered with the help of a hand-grinding mill and the power was sieved. The air-dried and powdered leaves were extracted successively with aqueous, ethanol and chloroform at 80^{o}C, 40^{o}C and room temperature respectively (Kokate, 1994; Owoyele et al., 2001). The dried extract was stored at 4^{o}C until use. The extract yields of the plants were 1.2, 3.0 and 2.0 g from 20.0, 30.0 and 20.0 g of powered leaves in 150 mL water, 300 mL methanol and 250 mL chloroform

respectively. The aqueous extract was dissolved in 0.9% saline while the methanol extracts and chloroform extract were dissolved in 2.5% Tween 80 and subsequently in normal saline.

Wister rats of either sex and of approximately the same age, weighting about 150-175 g were used for the study. They were housed in polypropylene cages are fed with standard clow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee and cleared by the same.

The animals were divided into control and test groups containing six animals each. The Control group received the vehicle (1% acacia) while the test groups received different extracts orally and were observed for mortality till 48h and the LD_{50} were calculated (Ghosh, 1994).

Anti-inflammatory activity was assessed by the method described by Winter *et al*. (1968). The rats were divided into eleven groups of six animals each. First group (negative control) received 1mL of normal saline, second group (positive control) received 10 mg/kg p.o. Diclofenac sodium, third, fourth and fifth groups were received aqueous, methanolic and chloroform extracts (100 mg/kg p.o.) of *Calotropis procera* respectively. Groups sixth, seventh and eighth were received aqueous, methanolic and chloroform extracts (100 mg/kg, p.o) of *Gymnema sylvestre* respectively. Groups ninth, tenth and eleventh were received aqueous, methanolic the chloroform extracts (100 mg/kg p.o) of *Hemidesmus indicus* respectively. After 1h, the rats were challenged with subcutaneous injection of 0.1 mL of 1% w/v solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmogrouph apparatus used for the measurement of rat paw volume was that of Singh and Ghosh (1968). The paw volume was measured immediately after injection (0h) and then first, second and third hour after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula, Percent inhibition = 100 (1-Vt/Vc), where 'Vc' represents edema volume in control and 'Vt' edeam volume in group treated with various plant extracts. The data were statistically analyzed through student's t-test.

RESULTS AND DISCUSSION

In the present study, anti inflammatory activity of aqueous, methanol and chloroform extracts of *C. procera*, *G. sylvestre* and *H. indicus* leaves were evaluated. From the results it was note that the extracts significantly ($P < 0.05$) inhibited the inflammatory edema, though

the inhibition was highest in methanolic extracts. The effect of methanolic extract was the same as that of 150 mg/kg of Diclofenac sodium, among the three tested plant species *H. indicus* showed the maximum inhibition. Extracts of *C. procera*, *G. sylvestre* showed a similar anti-inflammatory effect but lower than extract of *H. indicus*.

Carrageenan-induced rat paw edema has been used as an inflammation model in order to investigate the anti-inflammatory effect of drug (EI-Shenawy *et al*., 2002). Carrageenan induced inflammation is biphasic phenomenon. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action (Goodman and Gilman, 2001; Muthumani *et al*., 2010; Moulisha Biswas *et al*., 2011).

The result of the present study has shown that all the crude extracts of the investigated three plants exhibited very high anti-inflammatory activities. These activities may be linked with presence of polyphenolic compounds present in the extract. On preliminary phytochemical screening showed that the extract of *C. procera, G. sylvestre* and *H. indicus* contained anti-oxidative constituents, which includes flavonoids or flavonoidal compounds. Flavonoids found in many plants have been shown to have diuretic, laxative, antispasmodic, anti-hypertensive and anti-inflammatory actions (Owoyele *et al*., 2001; Nayak *et al*., 2006; Mule *et al*., 2008; Jain Ruchi *et al*., 2009; Madan *et al*., 2011; Amberkar *et al*., 2011).

From the results it has been concluded that the leaves of *C. procera, G. sylvestre* and *H. indicus* possess significant anti-inflammatory property in rats. There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation (Anderson *et al*., 1971; Shen, 1967; Weissmann, 1967; Jannoff and Zweifach, 1964). Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosomal membrane, which is one of the major events responsible for the inflammatory process (Nair *et al*., 1988). So, we can assume that our drug extract might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane. From the above studies it is quite apparent that the ethanolic extract possesses significant anti-inflammatory activity. The study justifies its use in inflammation as suggested in the folklore medicines. Further studies involving the purification of the chemical constituents of the plant and the investigation on the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and higher therapeutic index.

Table 1: Anti-inflammatory activity of aqueous extract of *C. procera, G. sylvestre* and *H. indicus* plants on carrageenan induced rats hind paw edema model

Treatment	Dose (Mg/Kg, P.O.)	Mean Paw Volume					% Inhibition of edema
		0 hr	1 hr	2 hr	3 hr	4 hr	
Control	00	9.0 ± 0.11	9.1 ± 0.13	10.3 ± 0.04	11.4 ± 0.12	11.6 ± 0.10	-
Standard (Diclofence sodium)	150	7.6 ± 0.02	7.3 ± 0.11	6.6 ± 0.16	5.9 ± 0.10	4.2 ± 0.11	63.79
Calotropis procera	100	6.3 ± 0.03	6.8 ± 0.10	6.4 ± 0.10	6.2 ± 0.17	5.6 ± 0.11	51.72
Gymnema sylvestre	100	7.7 ± 0.04	8.1 ± 0.12	7.4 ± 0.17	6.2 ± 0.18	5.2 ± 0.12	55.17
Hemidesmus indicus	100	7.5 ± 0.11	8.3 ± 0.12	7.2 ± 0.13	6.0 ± 0.01	5.5 ± 0.10	52.58

Table 2: Anti-inflammatory activity of ethanol extracts of *C. procera, G. sylvestre* and *H. indicus* plants on carrageenan induced rats hind paw edema model

Treatment	Dose (Mg/Kg, P.O.)	Mean Paw Volume					% Inhibition of edema
		0 hr	1 hr	2 hr	3 hr	4 hr	
Control	00	9.0 ± 0.11	9.1 ± 0.13	10.3 ± 0.04	11.4 ± 0.12	11.6 ± 0.10	-
Standard (Diclofence sodium)	150	7.6 ± 0.02	7.3 ± 0.11	6.6 ± 0.16	5.9 ± 0.10	4.2 ± 0.11	63.79
Calotropis procera	100	7.2 ± 0.11	8.1 ± 0.10	6.6 ± 0.11	6.4 ± 0.13	4.8 ± 0.22	58.62
Gymnema sylvestre	100	8.3 ± 0.15	9.8 ± 0.22	7.5 ± 0.17	7.3 ± 0.10	6.6 ± 0.10	43.10
Hemidesmus indicus	100	7.5 ± 0.10	8.4 ± 0.14	7.3 ± 0.11	6.1 ± 0.23	4.7 ± 0.14	40.51

Table 3: Anti-inflammatory activity of chloroform extract of *C. procera*, *G. sylvestre* and *H. indicus* plants on carrageenan induced rats hind paw edema model

Treatment	Dose (Mg/Kg, P.O.)	Mean paw volume					% Inhibition of edema
		0 hr	1 hr	2 hr	3 hr	4 hr	
Control	00	9.0 ± 0.11	9.1 ± 0.13	10.3 ± 0.04	11.4 ± 0.12	11.6 ± 0.10	-
Standard (Diclofence sodium)	150	7.6 ± 0.02	7.3 ± 0.11	6.6 ± 0.16	5.9 ± 0.10	4.2 ± 0.11	63.79
Calotropis procera	100	7.1 ± 0.12	6.9 ± 0.13	6.6 ± 0.10	6.0 ± 0.11	5.4 ± 0.15	53.45
Gymnema sylvestre	100	7.8 ± 0.16	6.7 ± 0.11	6.3 ± 0.02	5.6 ± 0.13	5.3 ± 0.11	54.31
Hemidesmus indicus	100	7.5 ± 0.04	6.4 ± 0.07	6.2 ± 0.10	5.3 ± 0.04	4.8 ± 0.10	58.62

ACKNOWLEDGEMENT

The authors thanks are due to Prof. S. Vidyadhar Principal Chebrolu Hanumaiah Institute of Pharmaceutical Sciences Guntur Andhra Pradesh for Post Graduate and Doctoral Extramural Research Programmes Scheme.

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Revised : 20.12.2011 *Accepted : 22.12.2011*