ANTI-INFLAMMATORY SCREENING OF SOME NOVEL COUMARIN DERIVATIVES

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Abstract-Inflammation is the primary host defense mechanism against all forms of injury. Excessive or inadequate activation of the system can have serious effects, as can the failure of inactivation mechanisms. Coumarins can reduce tissue edema and inflammation and inhibit prostaglandin biosynthesis, which involves fatty acid hydroperoxy intermediates. In this paper we would like to discuss the anti-inflammatory activity some substituted coumarin derivatives by means of LD₅₀ using Diclofenac sodium as standard.

Keywords- Coumarins, anti-inflammatory, Median Lethal Dose.

I. INTRODUCTION

Heterocyclic systems containing oxygen have occupied a unique place in the field of Heterocyclic compounds by their extensive natural occurrence and a wide variety of biological properties. Benzopyran 2-one or coumarin is a naturally occurring heterocyclic compound, the derivatives of which have exhibited anticoagulant, antimicrobial, anti-inflammatory, CNS activities. In recent years its industrial applications, photochemical behavior in solid state and its crystallography had gained importance.

Inflammation is a complex stereotypical reaction of the body expressing the response to damage of its cells and vascularized tissues and it has two main components – cellular and humoral. The cellular component involves the movement of leukocytes from blood vessels into the inflamed tissue. Various types of leukocytes are involved in the initiation and maintenance of inflammation. The discovery of the detailed processes of inflammation has revealed a close relationship between inflammation and the immune response.

Acute inflammation is mediated by granulocytes or polymorphonuclear leucocytes, while chronic inflammation is mediated by mononuclear cells such as monocytes and macrophages which can be further stimulated to maintain inflammation through the action of an adaptive cascade involving lymphocytes (T cells and B cells) and antibodies [1,2]. Like PGE₂, their biosynthesis is prevented by nonsteroidal anti-inflammatory agents (NSAIDs). NSAIDs are widely used for the treatment of pain, fever and inflammation [3, 4]. All of the NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy but also cause untoward side effects, in a significant fraction of treated patients and this frequently limits therapy.

Coumarin nucleus is the basis of various naturally occurring and synthetic compounds possessing several pharmacological and physiological activities. Plant extracts containing coumarin derivatives have been applied against gastrointestinal diseases, typhus and paralysis [5] for the therapy of leucoderma [6-7] and as anti co-agulants. In veterinary medicine they have been used as diuretics [8] and against trichomonas infection [9]. The potent antibiotics like novobiocin, coumermycin [10].

In view of the vital role played by coumarin in nature and the utility of its innumerable derivatives in the field of medicine, industry and agriculture, synthesis of coumarin derivatives have been undertaken during present work some of the coumarin derivatives have also been screened for their anti inflammatory and analgesic activity.
II. MATERIALS AND METHOD

Animals:
Swiss Albino mice of either sex (8-10 weeks old) weighing 22 to 30 gms and albino wister strain rats (180-200 gm) were used for the experiments. The animals were maintained under proper environmental conditions i.e., temperature 25±2°C and humidity 50 ± % with a 12 h light and dark period. They were housed in polypropylene shoe box type cages with stainless steel grill top, bedded with rice husk. The animals were provided with pelleted diet (Gold Mohur, Lipton, India) and water. Six animals were used in each control group and treated group.

Chemicals:
Compound was prepared in 2% aqueous Tween 80. Carrageenan type 4 was obtained from sigma co Ltd ( st, Louis, MD ). Acetic acid from Glaxo, acetyl salicylic acid from Ranbaxy, India. All other chemicals were of analytical grade and used as received.

Determination of Median Lethal Dose (LD50):
1. Mice (20-25gms) were kept for overnight fasting.
2. They were divided into groups of 6 each. The compound under study was injected by I.P. route or oral route in different doses to different groups.
3. The animals were then observed for signs of toxicity and the mortality for 2 hours and then at the end of 24 hours.
4. At the end of 24 hrs numbers of animals dead were counted in each group. Present death was then calculated and transferred probits using probit table. Probits were then plotted (Y-axis) against Log dose of the test compound.

Evaluation of Anti - Inflammatory Activity:
The albino (Wister strain) rats (180-200g), obtained from NIMHANS, Bangalore were used for the experiments. The rats were divided into four groups, each group consisting of six animals. Oedema was induced by subplantar injection of 0.1 ml 1% freshly prepared carrageenan into the right hind paw of each rat. The paw volume was measured before zero hr and at 3 hr after the injection of carrageenan using a plethysmometer, the test compound (100mg/kg, 200mg/kg, 300mg/kg, 400mg/kg) was administered intraperitonially to three group of rats. The fourth group of rats received Diclofenac sodium 100mg/kg (standard) for assessing comparative pharmacological significance. Drug pretreatment was given 1 hr before the injection of carrageenan. The percentage inhibition of oedema was calculated.

Table 1  LD50 value of 7-methyl-benzofuro (3,2-b) pyridino (4,3-d) -7- methyl-benzopyran - 3 - 2[H] one

| Group | Dose mg/kg | Log Dose | Dead total | Dead % | Corrected % | Probit |
|-------|------------|----------|------------|--------|-------------|--------|
| 1     | 1200       | 3.0792   | 6/6        | 100    | 93.75       | 6.532  |
| 2     | 1000       | 3.000    | 6/6        | 100    | 93.75       | 6.535  |
| 3     | 900        | 2.952    | 6/6        | 100    | 93.75       | 6.525  |
| 4     | 800        | 2.903    | 3/6        | 50     | 43.75       | 5.000  |
| 5     | 700        | 2.841    | 2/6        | 25     | 18.75       | 4.330  |
| 6     | 600        | 2.778    | 0/6        | 0      | 6.25        | 3.455  |
| 7     | 500        | 2.690    | 0/6        | 0      | 6.25        | 3.457  |
| 8     | 400        | 2.601    | 0/6        | 0      | 6.25        | 3.454  |
Correction for 0% dead = 100 (0.25/n) Correction for 100% dead = 100 x (n-0.25/n) Calculation:

Log Dose = 2.925

LD50 = 831.8 mg.

Table 2 LD 50 Value of 6- methyl - (S'-methyl-3'-phenyl-2'-benzo (b) furanyl) coumarin

| Group | Dose Mg/Kg | Log dose | Dead Total | Dead % | Corrected% | Probit |
|-------|------------|----------|------------|--------|------------|--------|
| 1     | 1000       | 3.00     | 6/6        | 100    | 93.75      | 6.532  |
| 2     | 900        | 2.94     | 6/6        | 100    | 93.73      | 6.535  |
| 3     | 800        | 2.901    | 6/6        | 100    | 93.75      | 6.532  |
| 4     | 700        | 2.845    | 6/6        | 100    | 93.75      | 6.535  |
| 5     | 600        | 2.778    | 3/6        | 50     | 43.75      | 5.000  |
| 6     | 500        | 2.690    | 2/6        | 25     | 18.75      | 4.330  |
| 7     | 400        | 2.602    | 0/6        | 0      | 6.25       | 3.455  |
| 8     | 300        | 2.471    | 0/6        | 0      | 6.25       | 3.457  |
| 9     | 200        | 2.301    | 0/6        | 0      | 6.25       | 3.45   |
| 10    | 100        | 2.000    | 0/6        | 0      | 6.25       | 3.4575 |

Correction for 0 % dead = 100 (0.25 / n)
Correction for 100 % dead = 100 x
Calculation:
Log Dose = 2.800

LD50 = 6.31.0 mg.

III. DISCUSSION

Different chemical structures have been found to possess different anti-inflammatory activities. Inflammation is a normal and essential response to any noxious stimulus, which threatens the host and may vary from a localized response to a more generalized one. In view of the complexity and multitude of biochemical factors involved in inflammatory events, few general correlations of chemical structures and physicochemical properties with biological activities would be expected. (LD50 values Shown in Tables 1, 2)

IV. CONCLUSION

Coumarin is the prototypical compound possesses anti-inflammatory activity. Naturally occurring coumarins and several fused coumarins isolated from plants extracts were found to be potent against inflammation and edema as well as against enzymes and chemical mediators implicated in the phenomenon of inflammation. LD50 value of 7-methyl-benzofuro (3,2-b) pyridino (4,3-d) -7- methyl-
benzopyran - 3 - 2[H] one and LD 50 Value of 6- methyl - (S’ -methyl-3'-phenyl-2'-benzo (b) furanyl) coumarin anti-inflammatory activity is reported in the form of Median Lethal Dose.

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