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Anti-inflammatory Activity and SAR of some Phenacyl Halide Derivatives of Piperidine 4-Carboxamide on Rat Hind-paw with Carrageenan-induced Edema.

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Abstract

Living bodies respond to foreign invaders by producing inflammatory actions which manifests multiple unwanted symptoms. A number of anti inflammatory compounds have been extracted and synthesized with none being the perfect choice. Three newly developed halogenated derivatives of piperidine-4-carboxamide were evaluated for anti-inflammatory activity. The anti-inflammatory activity was performed *in vivo* by inducing edema in rats' hind paw with carrageenan. All these three compounds exhibited considerable anti-inflammatory effects of varying degree. The % inhibition in edema volume produced by -chloro and -bromo derivatives of piperidine-4-carboxamide were potent and comparable to acetylsalicylic acid taken as standard while the -flouro derivative produced insignificant activity.

***Keywords*:**

Anti-inflammatory activity, carrageenan, post herpetic neuralgia, chemokines, piperidine 4-carboxamide.

1. INTRODUCTION

Inflammatory diseases are widely prevalent throughout the world. Potent anti-inflammatory activity has been reported in heterocyclic compounds such as derivatives of alkanoic acid, pyrimidine, thiazole, imidazole, benzimidazole, acridine, thiourea andthiazolidin(Dhingra AK *et al.,* 2017, Sondhi SM, *et al.,* 2007, Johar M *et al* 2002, Noe MC *et al.*, 2006). In an attempt to have anti-inflammatory agents, various workers synthesized piperidine derivatives with antagonist activity against tachykinin receptors and potent Nk_1/Nk_2 inhibitor and proved beneficial in the ailments such as inflammation, pain, depression, anxiety, post-herpetic-neuralgia, migraine, emesis, and also as anti-platelet agents (Ting PC *et al.,* 2001, Shen J *et al.*, 2017, Tahlan S *et al* 2017, Chen Y *et al,* 2013, Cao X *et al*., 2018, Elliott

M. *et al.*, 2002,Barta TE *et al.*, 2004).

Many of the piperidine derivatives synthesized as chemokine antagonists were used since decades to treat nephritis, demyelinating diseases, multiple sclerosis and still found effective as anti inflammatory, anti-angiogenesis, anti-arthritic (rheumatoid arthritis, osteoarthritis, osteoporosis), anticancer, antibacterial and antifungal agents(Spector R. *et al.,* 1979,Aloip J-C et al., 1998,Soni *et al.*, 2017, Martins P *et al*., 2015, Rafuq K, *et al.,* 2013). Among heterocyclic compounds, piperidine-4-carboxamide (Isonipecotamide) is well known nitrogen containing six-member heterocyclic compound. The derivatives of isonipecotamide hold a wide range of biological and physiological actions such as antimicrobial, antitumor, analgesic and antiarthritic (Nugiel DA *et al.*, 2002, Ask A-L *et al*., 1998, Ivanoc C *et al*., 1995). Keeping in view these potentials, the synthesis of various substituted derivatives of isonipecotamide were reported by our group with multiple biological activities (Rauf A *et al.*, 2016, Akhtar S *et al*, 2003). The work was extended to explore the anti-inflammatory potential as well which was assessed by using Carrageenan induced rat hind paw edema method. In this research article three halogenated derivatives of isonipecotamidehave been focused to describe the structure-activity relationship (SAR) as anti-inflammatory agents. The study also discerns the significance of nature of substituents in the halogen family.

2. MATERIALS AND METHOD:

Materials:

Derivatives of Piperidine-4-carboxamide

Phenacyl halide derivatives of piperidine-4-carboxamide were prepared via partial organic synthesis by refluxing piperidine-4-carboxamide with respective phenacyl halides in the presence of acetone. The obtained products were characterized using spectroscopic techniques. The obtained derivatives 4-carbamoyl-1-[(4-bromophenyl)-2-oxoethyl]-piperidinium bromide, 4-carbamoyl-1-[2-(4-chlorophenyl)-2-oxoethyl]- piperidinium chloride, and 4-carbamoyl-1-[2-(4-fluorophenyl)-2-oxoethyl]-piperidinium bromide were coded as compound V, VI and VII respectively (Akhtar S *et al.*, 2003).

Animals

Wistar rats weighing 250-400 g were utilized for assessment of anti-inflammatory activity. The animals belonged to either sex and were reared at animal house in PCSIR Laboratories Pakistan Ltd. Each group of seven animals was maintained under standard colony condition i.e., 12 hours dark and 12 hours light cycle at a temperature of 30 ± 2C, fed with balanced diet and water.

Chemicals

Aspirin 300 mg (Acetylsalicylic acid) and the synthesized compounds in the same dose were homogenized with appropriate quantities of 1.5% aqueous gum like tragacanth. These suspensions were administered (5 ml/kg body weight) to the test animals orally by intubation. Carrageenan was used for inducing inflammation. The anti inflammatory assessment was conducted by computing mean rise in volume of hind paw after subplantar dose of carrageenan (Winter CA *et al*., 1962).

Method:

Animals were grouped as test, control and standard comprising seven rats each. The injectable solution was prepared by dissolving 0.1 ml 1% carrageenan in 0.9% saline (Sigma, USA). Animals were injected in the right-hind-paw under the plantar aponeurosis (25).

A dose of test compounds at 300mg/kg of body weight was orally administered to test group one hour before the carrageenan injection. Same volume of saline was given to the control group while group of standard was injected with acetylsalicylic acid (Aspro, Nicolas Pakistan Ltd.) in the same manner through the same route as that of the test compounds.

The inflammation was quantified in terms of mL using a plethysmometer (7150 Ugo Basile) immediately, before carrageenan injection and then 1, 2, 3, 4, 5 and 6 hours after carrageenan injection. The percentage of inhibition in each group was computed by comparing edema in test andcontrol groups.

The anti-inflammatory action was enumerated by the subsequent relationship (Palanichamy *et al*., 1990):

paw volume of control - paw volume of drug treated / paw volume of control * 100

STATISTICAL ANALYSIS

The anti-inflammatory action outcomes were stated as an average increase in the volume of paw±S.E.M. in terms of milliliters. The significant mean difference was established by student's t test. Values falling below $P<0.05$ were considered significant and those below $P<0.01$ highly significant. All statistical investigations were carried out according to the method of Alcaraz and Jimenez (Alcararaz M *et al.,* 1989).

3. RESULTS AND DISCUSSION The results of the study showing anti inflammatory effects of these halogenated derivatives by edema production in volume (ml) were presented in Table-1 and Figure-1. The anti-inflammatory effects were indicated as percent inhibition of edema by aspirin

(acetylsalicylic acid) taken as standard and the test compounds on carrageenan induced paw edema in rats.

The anti-inflammatory activities of halogenated derivatives of isonipecotamide (V, VI and VII) had been demonstrated in Table-1. The corresponding figures were showing the subsequent change in carrageenan induced edema volume in compound treated animals. All the compounds were showing anti-inflammatory effects of varying degrees. The bromo and *chloro* derivatives (V and VI respectively) were showing the same effects after the 3rd hour of edema production. The time of onset of action was early in chloro derivative as compared to that of bromo derivative which initiated edema inhibition at 3rd hour reaching to maximum activity gradually i.e., 81.8 ± 13.0, 81.10 ± 11.6 and 91.9 ± 5.4 at 4th, 5th and 6th hours respectively. The chloro derivative showed highly significant % inhibition of 81.8 ± 13.0 to 91.9 ± 5.4 (Table 1) at 4th to 6th hours as compared to aspirin where percent inhibition was 50.0 ± 11.2, 51.4 ± 13.4 and 37.8 ± 4.5 at 4-6 hours respectively. The effects in case of bromo compound were highly significant in the later stages. Comparing the percent inhibition of chloro derivative (VI) to aspirin, it could be seen that compound VI was producing the

same degree of inhibition at 4th hour showing more promising effects at 5th and 6th hours i.e., 73 ± 18.1 and 75.7 ± 11.6 while for aspirin, it was 51.4 ± 13.4 and 37.8 ± 4.5 at the same hours. The behavior of flouro-derivative (VII) was quite different from both chloro and bromo derivatives. Edema was highly significant at 3rd hour and then at 4th, 5th and 6th hours, significant increase in edema was present as compared to control. Anti-inflammatory effects of compound VII were not better than aspirin, however at 6th hour, % inhibition of aspirin and compound VII were found to be the same.

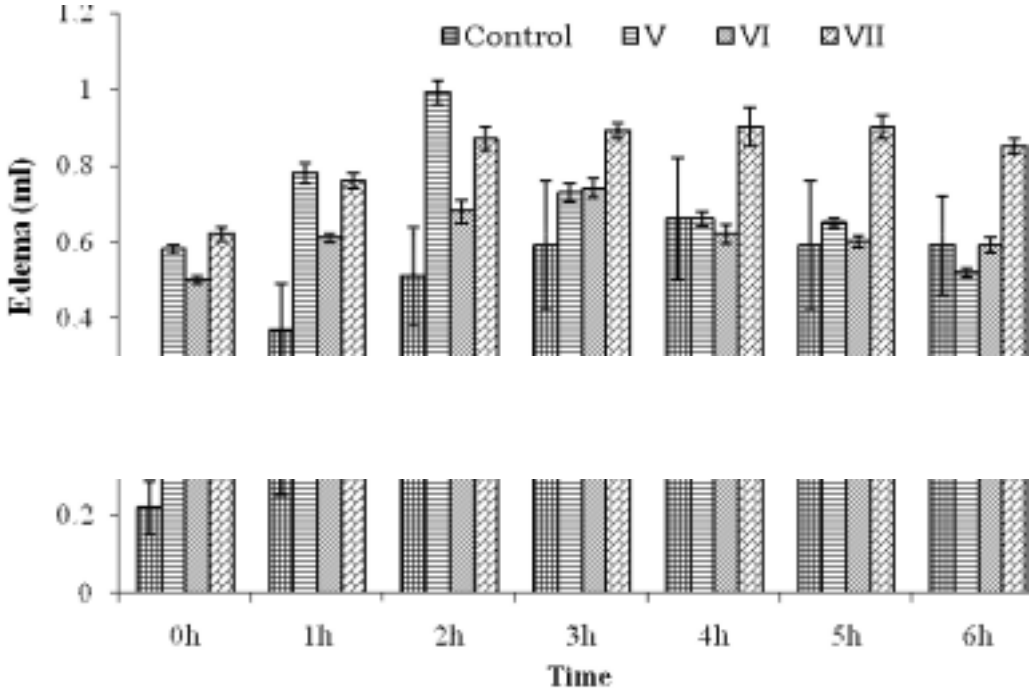

Fig 1: Anti-inflammatory activity of isonipecotamide derivatives (V, VI, VII)

The variation in results might be correlated and the established the mechanism of anti inflammatory effects considering the following facts:

Inflammation is a defense response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane J. *et al*., 1995, Delporte C. *et al*., 1996). Inflammation can be induced by two types of stimuli i.e., immunological (antigen induced) and non-immunological (carrageenan induced). In case of non-immunological model of inflammation various stimuli such as carrageenan, tragacenth and zymosan can be used to induce edema (Rao TS *et al.,*1994). Carrageenan induced inflammation occurs in initial and late phases. The initial phase occurs between 0 to 2.5 hours, during this phase histamine, 5-hydroxy tryptamine (5HT) and bradykinin are predominantly released. The late phase starts due to the over production of prostaglandins such as PGG_2, PGH_2 and PGE_2, in tissue (Smit H *et al*., 2000, Di Rosa 1974 *et al*., 1974). Assuming that at least a part of these processes is subject to inhibition by these newly synthesized compounds, therefore this method has been chosen for this investigation.

Carrageenan induced rat paw edema test is widely used to study both steroidal and non steroidal anti-inflammatory drugs (Mantri P. *et al*., 1994). Prostaglandins (particularly prostaglandin E_2) and leukotriene (LTB_4) are

the metabolites of arachidonic acid. . This arachidonic acid is located within the phospholipids of cell membrane and plays a key role in the process of inflammation. Prostaglandins and leukotrienes being inflammatory mediators are produced via cyclo-oxygenase and lipoxygenase pathways respectively. Therefore, inhibitors of these two pathways such as aspirin (Cyclooxygenase inhibi-

tor).and caffeic acid (lipoxygenase inhibitor) have potential therapeutic value (Mazzanti G *et al*., 1994, Singh S. *et al*., 1996). This method allows screening of hundreds of chemicals in a relatively short period (Kalant H *et al.,* 1989). Thereby development of afore-mentioned inhibitors is of considerable importance (Salmon JA *et al.,* 1987).

In the present study carrageenan was used to produce edema in rats to evaluate anti inflammatory activities of the halogenated compounds. The anti-inflammatory effects thus produced given in Fig. 1 demonstrated the time dependent change in edema volume after the sub plantar administration of 1% carrageenan.

Among the three halogenated derivatives, it was evident that the presence of F in the phenyl ring could not succeed to produce significant % inhibition in edema. Indeed, carrageenan induced paw edema is mainly characterized by the pivotal role of prostaglandins release (Panthong A *et al.*, 1989). Therefore, the most frequently encountered mechanism of action amongst anti-inflammatory drugs is the inhibition of prostaglandin synthesis due to the inhibition of cyclo-oxygenase enzyme (Lino C *et al*., 1997, Smith J *et al.,* 1971).

4. CONCLUSION:

In this study it can be hypothesized that the inhibition of edema caused is possibly due to the inhibition of prostaglandins. Moreover, this study offers valuable insight into structure-activity relationships of the derivatives of piperidine having different functional groups at different positions in the benzyl ring and may lead to the development of a new class of drugs. Synthesized compounds are promising anti-inflammatory agents however, drug discovery process requires in-depth evaluations of toxicity and suitability profiles.

Declarations

Ethics Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent for publication

Not applicable

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author’s contributions

SA and MA synthesized the new derivatives, RAK provided necessary support for animal studies, NM and AA provided support in synthesis design and RI helped in writing

manuscript.

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