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PHARMACOLOGICAL ACTIVITY OF MORPHOLINO COMPOUND

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The pharmacological effect of morpholino compound in different biological field is of great importance for researchers and investigators. They are working day and night to synthesize pharmacologically more effective morpholino derivatives with less toxic effects. This kind of pharmacological screening of synthesized compounds can lead to the discovery of biologically useful compounds. The findings of the present study are encouraging, since present compound showed a promising analgesic activities and anti-inflammatory. Chemically synthesized morpholino compound are tested for its analgesic and inflammatory activities in intact albino mice ad rat. Compound was administered orally in different doses using the tail immersion test in mice for analgesic activity. The results of this study demonstrated that compound has inhibitory effect on arachidonic acid metabolisms via cyclooxygenase pathway or we can say the inhibition of prostaglandins biosynthesis. Classical non-steroidal anti-inflammatory drug, which inhibits cyclooxygenase, can shift arachidonic metabolism to lipoxygenase products. These findings reflect a new fact of pharmacological action of this compound like inhibition of arachidonic acid pathway *in vivo*. It can be concluded that the present study revealed a fair success rate but it is necessary to carry out further investigation to confirm the results.

Keywords: Analgesic, anti-inflammatory, morpholino compound.

INTRODUCTION

Synthetic chemical compounds are having the history to produce greater changes in lives. Any minute change in their structures or addition and suppression in existing structures may reveal different pharmacological effects for this running world therefore scientists are day and night proving and evaluating the previous and new changes in this field. A large number of analgesics are synthesized and used widely for the treatment of different types of pain with considerable success. Scientific studies are, therefore required to judge their actual efficacy, mode of action, and other limitations to widen the scope of the use of new synthetic compounds (Shi, *et al.,* 2002). The present study has yielded a fair success rate. This kind of pharmacological screening of new synthesized compound can lead to the discovery of useful biological activity. The findings of the present study are encouraging as morpholino compound showed a significant analgesic effect.

The results of the experiments conducted with morpholino compound revealed significant pharmacological activity. It is encouraging to find out significant activity of new synthetic compound and the work could be extended to study further pharmacological effects of the compound.

MATERIALS AND METHODS

Synthetic compound (2-(3'-methoxy-4-hydroxyphenyl)-3-(4-N-morpholino phenyl)-1,3-thiazolidine-4-one) was provided by Dr. Zafar Saeed Saify, Faculty of Pharmacy, University of Karachi. The purity and identity of the compound was confirmed by UV, NMR and MS studies. Other chemicals used were gum tragacanth and acetylsalicylic acid

Animals

Albino mice and rats of either sex obtained from PCSIR Laboratories Complex, Pakistan were used to determine the analgesic and anti-inflammatory activities. Mice weighing 25-30g and rats between 160-210 were used in colony cages (seven animals in each group) with access to food and water. They were maintained in a climate and light controlled room ($30^oC \pm 1^oC$ 12/12 hours light/dark cycle) at least 7 days before testing or administration of the drug.

Method for assessment of analgesic activity: tail flick latency test

In this investigation analgesia was assessed by tail immersion method Luiz *et al.,* 1988. Mice were held in a suitable restrainer with the whole tail extending out. The distal end of the tail (2-3 cm) was immersed in the water bath thermostatistically maintained at 51^oC.The reaction time (in seconds) to flick the tail from the water was recorded as tail flick latency before the administration of any drug as pre-drug tail flick latency and then recorded at the time interval of +30, +60, +90, +120, +150, +180 and +210 minutes after the administration of the test drug (post-drug tail flick latency) and the standard drug. The maximum immersion time of 30 seconds was maintained to avoid the thermal injury of the tail. All the drugs were administered orally in the doses of 50, 75 and 100 mg/kg. The test compound was administered as a suspension in 1% gum tragacanth. The control animals received only the vehicle.

The criterion of analgesia was post drug latency, which was greater than two times the pre-drug average latency. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by the test and standard drugs. Analgesia TFLD was calculated as follows:

Analgesia TFLD = Post drug tail flick latency – Pre drug tail flick latency.

Writhing test

The test was accomplished according to the modified method of Koster *et al*., 1959. The writhes were induced by intraperitonial administration of the acetic acid solution 10ml/kg. Thirty minutes prior to the administration of the acetic acid, the animals were treated orally with the test substance. Numbers of writhes were counted for 20 min. immediately after acetic acid. A reduction in the number of writhing as compared to the control group was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhing.

Anti-inflammatory testing

In present study, anti-inflammatory activity was determined in rats by measuring the mean increase in hind paw volume after the sub plantar injection of carageenan (Winters *et al*., 1962). All rats were divided into five groups, each group having seven rats. The animals were injected with 0.1 ml 1% carageenan in 0.9% saline (Sigma, USA) in the right hind foot under the plantar aponeurosis.

The test groups were given orally 50, 75 and 100 mg/kg of synthetic compound before the carageenan injection. The controls were given the same volume of saline. Another group of rats was treated with 300 mg/kg of Aspirin orally 1h before carageenan injection. The inflammation was quantitated in terms of milliliters using plethysmometer (7150 Ugo Basile) immediately before carrageenan injection. The percent inhibition of edema was calculated for each group with respect to its vehicle-treated control group. The inflammatory activity was calculated by the following relationship:

$$\frac{A-B}{A} \times 100$$

(Palanichamy and Nagarajan, 1990). Where A and B denote mean increase in paw volume of control and drug-treated animals respectively.

Statistical analysis

Values for activities were expressed as mean after drug administration ± SEM. The significance of difference between means was determined by Dunnett's t-test and values of $P<0.05$ were considered significant and $P<0.01$ as highly significant. All statistical procedures were performed according to the method of Alcaraz and Jimenez (1989).

RESULT

Table 1 shows the analgesic activity of the compound by tail flick test. It reveals significant analgesic activity at all doses but it is highly significant at 100 mg/kg as compared to reference drug aspirin. Table 2 shows the result of writhing test which are highly significant and comparable with reference drug at all doses. Table 3 shows the results of anti-inflammatory activity of the compound. It exhibits significant anti-inflammatory activity at all doses, which is similar to reference drug aspirin.

DISCUSSION

Drug designing is a very pronounced concept used in therapeutic disciplines. Its usefulness lies in its innovative approach towards the development of potent, safe and effective compound of biological interest. Taking into considerations all parameters that form the basis of drug designing, a variety of potent analgesic and anti-inflammatory compounds have been prepared in the past. Salicylic acid was first non-steroidal anti-inflammatory agent, then phenylbutazone in 1952 and then mefenamic acid; ibuprofen and indomethacin were introduced (Fareed et al., 2005). The major problems encountered by these agents are their toxic tendencies and respiratory depression profiles.

In the present study attempt has been made to check the pharmacological aspects of morpholino compound which exhibit significant analgesic and anti-inflammatory activities at all doses.

Pain can be relieved centrally as well as peripherally (Curtis and Curtis, 1990). Generally there are two major types of peripheral acting analgesics, those that prevent the activation (sensitization) of the nociceptors and those, which directly down regulate activated (sensitized) nociceptors (Ferreira 1990). NSAIDs like aspirin inhibit synthesis of prostaglandin, which make the nociceptors more sensitive to pain producing agents. Such as bradykinin (Curtis and Curtis 1990). The observation that the morpholino compound induced a profound inhibitory effect is dose related and was comparable to that elicited by aspirin. The results of tail flick latency differences are comparable with aspirin, its initial response was achieved within +30 minutes it means it has a rapid onset of action and most probably follows the mechanism of NSAIDs.

The results reported in this study demonstrated that compound has inhibitory effect on arachidonic acid metabolisms via cyclooxygenase pathway. The finding is further corroborated by our result that compound inhibit the carrageenan induced inflammation in rat paw.

Table 1
Analgesic effect of 2-(3'-methoxy-4-hydroxyphenyl)-3-(4-N-morpholino phenyl)-1, 3-thiazolidine-4-one in mice tail immersion method

Treatment	Dose mg/kg orally	Analgesia TFLD or mean increase in latency after drug administration ± S.E.M.(s)						
		+ 30	+ 60	+ 90	+ 120	+ 150	+ 180	+ 210 (min)
Control		2.0 ± 0.014	2.1± 0.012	2.1 ± 0.014	2.0 ± 0.014	2.0 ± 0.020	2.0 ± 0.021	2.0 ± 0.021
2-(3'-methoxy-4-hydroxy- phenyl)-3-(4-N-morpholino phenyl)-1, 3- thiazolidine-4-one	50	1.0 ± 0.011*	1.1 ± 0.011*	1.4 ± 0.015*	1.5 ± 0.023*	1.8 ± 0.013*	1.8 ± 0.018*	1.9 ± 0.021*
	75	1.7 ± 0.013*	1.8 ± 0.021*	2.0 ± 0.023*	2.0 ± 0.013*	2.2± 0.018*	2.2 ± 0.018*	2.3 ± 0.018*
	100	2.0 ± 0.012*	2.1 ± 0.020*	2.2 ± 0.024**	2.3 ± 0.023**	2.4 ± 0.013**	2.4 ± 0.013**	2.6 ± 0.013**
Aspirin	50	2.0 ± 0.012*	2.1 ± 0.012*	2.2 ± 0.012*	2.3 ± 0.012**	2.4 ± 0.023*	2.5 ± 0.012*	2.6 ± 0.012*
	75	2.4 ± 0.012*	2.5 ± 0.011**	2.6 ± 0.011*	2.7 ± 0.011*	2.8 ± 0.011*	2.9 ± 0.011*	3.0 ± 0.011*
	100	2.9 ± 0.011*	3.0 ± 0.011*	3.1 ± 0.011*	3.2 ± 0.01*	3.3 ± 0.011*	3.4 ± 0.011**	3.4 ± 0.012*

Mean ± S.E.M, n=7, Significance relative to control = *P < 0.05, ** P < 0.01.

Table 2
Effect of 2-(3'-methoxy-4-hydroxyphenyl)-3-(4-N-morpholino phenyl) -1, 3- thiazolidine-4-one and aspirin on acetic acid induced writhing in mice.

Treatment	Dose mg/kg	No. of writhes	Inhibition (%)
Control		146 ± 0.26	-
2-(3'-methoxy-4-hydroxyphenyl)-3-(4-N-morpholino phenyl)-1, 3-thiazolidine-4-one	50	64 ± 0.20**	56.16%
	75	50 ± 0.18***	65.75%
	100	42 ± 0.31**	71.23%
Aspirin	50	62 ± 0.14**	57.24%
	75	49 ± 0.22*	66.20%
	100	42 ± 0.29***	71.03%

Mean ± S.E.M, n=7, Significance relative to control = *P < 0.05, ** P < 0.01 and ***P<0.005.

Classical non-steroidal anti-inflammatory drug, which inhibits cyclooxygenase, can shift arachidonic metabolism to lipoxygenase products.

These findings point to a new aspect of pharmacological action of this compound like inhibition of arachidonic acid pathways in vivo so that may exert analgesic or anti-inflammatory activity where arachidonic acid metabolites are implicated.

CONCLUSION

The results of morpholino compound revealed that the present study has yielded a fair success rate. This kind of pharmacological screening of new synthesized compound can lead to the discovery of useful biological activity.

During the course of present work it is observed that compound possess significant pharmacological activity and hence work should be extended to study toxicological effects of the compound.

Table 3
Anti-inflammatory effects of 2-(3'-methoxy-4-hydroxyphenyl)-3-4-N-morpholinophenyl)-1,3-thiazolidine-4-one on carrageenan-induced rat paw edema

Treatment	Dose mg/kg P.O.	Before carra-geenan	Mean paw volume ± S.E.M. (ml)					% Inhibition in edema ± S.E.M. (%) (ml)				
			+ 1 h	+ 2 h	+ 3 h	+ 4 h	+ 5 h	+ 1 h	+ 2 h	+ 3 h	+ 4 h	+ 5 h
Control		0.81 ± 0.0011	1.34 ± 0.0014	1.56 ± 0.0012	1.61 ± 0.0031	1.94 ± 0.0011	1.79 ± 0.0017	-				
2-(3'-methoxy-4-hydroxy-phenyl)-3-4-N-morpho-linophenyl)-1, 3-thiazolidine -4-one	50	0.81 ± 0.0014	1.10 ± 0.0018	1.12 ± 0.0020	1.13 ± 0.0018	1.11 ± 0.0042	1.14 ± 0.0024	45.28 ± 0.08	58.66 ± 0.18	60 ± 0.25	73.45 ± 0.24	66.32 ±0.16
	75	0.80 ± 0.0012	1.99 ± 0.0021	0.97 ± 0.0027	1.10 ± 0.0026	1.11 ± 0.0020	1.12 ± 0.0032	64.15 ± 2.08	77.38 ± 2.15	62.5 ± 0.06	72.56 ± 0.47	67.34 ±2.23
	100	0.81 ± 0.0011	0.97 ± 0.0032	0.99 ± 0.0020	0.95 ± 0.0024	1.10 ± 0.0026	1.13 ± 0.0021**	69.81 ± 1.15	76 ± 1.90	82.5 ± 2.68	74.33 ± 2.42	67.34 ±1.08
Aspirin	50	0.81 ± 0.0011	0.99 ± 0.0011	1.07 ± 0.0011	1.09 ± 0.0012	1.17 ± 0.0020	1.11 ± 0.0011	66.03 ± 0.81	662 ± 0.85	65 ± 0.69	68.4 ± 0.75	70.5 ±0.96
	75	0.80 ± 0.0022	0.99 ± 0.0011	1.02 ± 0.0011	1.09 ± 0.0012	1.11 ± 0.0021	1.08 ± 0.0011	64.1 ± 0.09	71.42 ± 0.93	63.7 ± 1.12	72.8 ± 1.1	72.5 ±1.09
	100	0.80 ± 0.0011	0.95 ± 0.0012	0.97 ± 0.0011	0.99 ± 0.0020	1.07 ± 0.0021	1.09 ± 0.0021	71.6 ± 0.67	77.9 ± 1.35	76.2 ± 1.48	76.3 ± 1.51	71.56 ±1.81

Mean ± S.E.M
n=7
Significance relative to control = *P < 0.05, ** P < 0.01

REFERENCES

Alcaraz MJ and Jimenez MJ (1989). Anti-inflammatory compound from *Sideritis javalambrensis* n-hexane extract. *J. Nat. Prod.,* **52**: 1088-1091.

Curtis SM and Curtis RL (1990). *In*: Pathophysiology, Concepts of Altered Health States, Somatosensory function and pain. C.M.J.B. Porth (Ed.) 3rd edn., Lippincott Company, Philadelphia, pp.840-872.

Fareed Hasan SM, Tasneem A, Nasira T and Fouzia H (2005) Pharmacokinetics of diclofenac sodium in normal man. *Pakistan journal of Pharmaceutical Sciences,***18**(1): 18-24.

Ferreira SH (1990). *In*: Migraine a Spectrum of Ideas, A classification of peripheral analgesics based upon their mode of action. Sandler M and Collins G (Eds.), Oxford Medical Publications, New York, pp.35-80.

Koster R, Anderson M and De Bear EJ (1959). Acetic acid for analgesic screening. *Fed. Proceed.*, **18**: 412-416.

Luiz CDS, Mirtes C, Sigrid LJ, Mendacolli M, Cecilia G and Gustaf T (1988). Screening in mice of some medicinal plants used for analgesic purposes in the state of Sao Paulo. *Journal of Ethnopharmacology*, **24**: 205-211.

Palanichamy S and Nagarajan S (1990). Analgesic activity of Cassia Alata leaf extract and Kaempferol-3-0-sophoroside. *Fitoterapia,* **LXI**: 44-47.

Shi M, Wu XF and Rong G (2002). C(2)-Symmetric Dialkoxyphosphoramide and dialkoxythiophosphoramide derivatives of (1R, 2R)-1,2-diaminocyclohexane as chiral ligands for the titanium (IV) alkoxide-promoted asymmetric addition reactions of diethylzinc to arylaldehydes. *Chirality,* **14**(1): 90-95.

Winter CA, Risley EA and Nuss GW (1962). Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med*., **111**: 544-547.

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IN VITRO AVAILABILITY OF LOMEFLOXACIN HYDROCHLORIDE IN PRESENCE OF ESSENTIAL AND TRACE ELEMENTS

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Lomefloxacin hydrochloride is a third generation fluoroquinolone antibacterial agent having a broad spectrum of action against a wide range of Gram-positive and Gram-negative organisms. The *in vitro* availability studies of lomefloxacin were carried out in presence of essential and trace elements such as magnesium, calcium, chromium, ferric, ferrous, cobalt, nickel, copper, zinc and cadmium in simulated gastric juice, simulated intestinal juice and blood pH at 37^{o}C using B.P 2003 dissolution test apparatus. It was observed that availability of lomefloxacin was depressed in presence of nickel and zinc in simulated gastric juice and in presence of Fe^{2+} in simulated intestinal juice, while many metals like magnesium, chromium, iron (both Fe^{2+} and Fe^{3+}), cobalt, nickel, copper and cadmium depressed the availability of lomefloxacin at blood pH. Furthermore, the availability of lomefloxacin alone in simulated intestinal juice and at blood pH was reduced as compared to simulated gastric juice. The antibacterial activities of lomefloxacin in presence of these metal ions were observed and compared to control against six different microorganisms i.e., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus fragilis* and *Streptococcus pneumonia* by disc diffusion method to measure the inhibitory zone and MIC were determined by tube dilution method.

Keywords: Lomefloxacin, hydrochloride, *in vitro* availability, antibacterial activity.

INTRODUCTION

Lomefloxacin hydrochloride is 1- ethyl 6,8-difluoro-1,4-dihydro 7-(3-methyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid monohydrochloride (Colin., 1999) shows the attachment of C-6 fluorine atom contributes to the antibacterial activity and its binding to DNA gyrase (topoisomerase II) (Domagala *et al*., 1986, Koga *et al*., 1980). It exert its antibacterial action by antagonism of the enzyme DNA gyrase also known as topoisomerase II, causing inhibition of DNA synthesis, antagonism of RNA and protein synthesis and ultimately cell death (Wolfson *et al*., 1989).