doi.org/10.36721/PJPS.2021.34.5.SUP.1983-1988.1

Moxifloxacin and *gemifloxacin* mediates its antispasmodic profile via ATP-sensitive potassium channels: An *in-vitro* bioassay study

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Abstract: *Moxifloxacin* and *gemifloxacin* were tested on isolated rabbits' jejunal preparations as little is known about its effects on gastrointestinal tissues. *Moxifloxacin* and *gemifloxacin* were tested in concentrations 0.01-10µg/mL for possible effect(s) on isolated rabbits' jejunal preparations. The drugs were applied on spontaneous, on low K^+ (20mM)-induced contractions and on high K^+ (80mM)-induced contractions. Response was plotted as % of its respective controls. EC_{50} for *Moxifloxacin* and *Gemifloxacin* on spontaneous (without *Glibenclamide*) contractions are 2.83±0.5µg/mL and 1.11±0.2µg/mL, respectively. *Moxifloxacin* and *Gemifloxacin* relaxed the low K^+ (20mM) -induced contractions, which were inhibited in presence of *Glibenclamide* (3µM). Our result indicates that the relaxant activity of *Moxifloxacin* and *Gemifloxacin* is mediated possibly through activation of ATP-sensitive potassium channels (K_{ATP}). The relaxant effect of *Moxifloxacin* and *Gemifloxacin* is predominantly mediated by activation of ATP-Sensitive potassium channels (K_{ATP}), which could be cause of one of relaxing mechanisms.

Keywords: *Moxifloxacin*, *gemifloxacin*, ATP sensitive K^+ channels, K^+ channels activation, nicorandil.

INTRODUCTION

Fluoroquinolones (FQs) are broad spectrum antibiotics that are used for treatment of wide range of infections caused by susceptible strains of microorganisms (Oliphant and Green, 2002, Park-Wyllie *et al*., 2006). Newer FQs have been introduced into the market for the last 20 years (Emmerson and Jones, 2003). FQs are synthesized in laboratory by modification of the fluoro-group at position no-6 of *Nalidixic acid* that has widely improved its anti-gram-positive bacterial activity (Emmerson and Jones, 2003). *Ciprofloxacin* and *norfloxacin* are commonly used for the treatment of the complicated urinary tract infections (UTIs) these days. Newly introduced *moxifloxacin* is famous for the treatment of complicated and un-complicated respiratory tract infections (Liu, 2010). Besides its therapeutic uses, FQs are also associated with adverse drug reactions (Chen *et al*., 2000). Some of the quinolones have been withdrawn from the market due to severe adverse drug reactions, which have raised questions about the safety of the quinolones (Kahn, 2000). The serious adverse effects noted with the use of FQs include tendinitis and tendon rupture, prolongation of QT interval and dysglycemia (Ahmad *et al*., 2014). *Trovofloxacin* was withdrawn from market due to causing severe hepatitis while *sparfloxacin* was withdrawn from market due to reports of torsade de pointes and severe phototoxicity (Chen *et al*., 2000, Tokura *et al*., 1996). Similarly, *temofloxacin* and *levofloxacin* have limited use these days due to its phototoxic and CNS related adverse effects as well as causing hemolysis of RBCs (Lawrence *et al*., 2006). FQs cause the secretion of insulin via blockage of ATP-sensitive potassium channels in pancreatic β-cells (Saraya *et al*., 2004). *Moxifloxacin* was approved by US FDA in 1999 (Barrett, 2000). In animal studies, *moxifloxacin* has produced blood glucose homeostasis abnormalities in all diabetic rats (Ambadasu *et al*., 2017). Canadian Adverse Drug Reaction Monitoring Program (CADRMP) reported that *gatifloxacin, levofloxacin* and *moxifloxacin* are under Metabolic and Nutritional Disorders category (Nasr *et al*., 2017). Since little is known about effects of *moxifloxacin* and *gemifloxacin* on rabbits' gastrointestinal tissues, hence, the current study was carried out to get a clear picture.

MATERIALS AND METHODS

Drugs and Chemicals

Moxifloxacin, *gemifloxacin*, *glibenclamide* and *nicorandil* were respectively purchased from Getz Healthcare Pharmaceutical Karachi, Sami Pharmaceutical Karachi, Sanofi Aventis Healthcare Pharmaceutical and Bayer AG Germany Healthcare Pharmaceutical, Karachi. Other chemicals were of analytical grade. *Moxifloxacin, gemifloxacin, nicorandil* and *verapamil* were solubilized in distilled water while *glibenclamide* was solubilized in 10% DMSO. These vehicles used to solubilization of test materials have no influence on the spontaneous contractions of the smooth muscles being studied in the

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experiments. The test concentrations of the drugs were in range of 0.01-10µg/mL for this *in-vitro* studies.

Experimental Animals and their food

Adult rabbits (1.5-2.5kg) of either sex was breeded at the animal house of Pharmacy Department, Shaheed Benazir Bhutto University, Sheringal, Dir Upper, Khyber Pakhtunkhwa, Pakistan. The animals were maintained on 23-25^{O}C in a relative % humidity ranging 45-55. They were feed on standard diet of pellets. The animals had free access to fresh tap water. The studies were approved by the Research Ethics Committee of Shaheed Benazir Bhutto University, Sheringal under notification No: SBBU/IEC-20-01**.** Before starting the activity, the rabbits were acclimatized for seven days. The animals were exposed to 12 hours light and 12 hours dark cycle. Experiments were performed according to the standard guidelines outlined in the "Animals Bye-Laws 2008 (Scientific Procedures Issue- 1) of the University of Malakand (Ali *et al*., 2009).

Data Recording

Intestinal responses of rabbits' jejunal preparations were recorded on 4 channels Power-Lab Model: 4/25T, AD Instruments, made in Australia. The data acquisition system was coupled with Force Transducer through a Bridge amplifier (FE 221) having Model no, MLT 0210/ A Pan Lab S.I. For interpretation of recorded data, Lab Chart 7.3.8 was used as per our previous reported data (Ali *et al*., 2009, Ali *et al*., 2011a).

In-Vitro Bioassays

The experiments were conducted as per approved procedures (Ali *et al*., 2011b, Ali *et al*., 2017)

Rabbits' isolated Jejunal Preparations

Moxifloxacin and *gemifloxacin* were conducted on the rabbits' jejunal spontaneous contractions as per standardized protocols (Ali *et al.,* 2017, Gilani *et al*., 1994). Briefly describing, the rabbits were kept overnight on fasting. Rabbits were slaughtered and their abdomens were opened through a midline incision. The jejunums were obtained. All the mesenteries were removed carefully. Small portions ranges from 1.5-2.0cm parts of jejunums were prepared (Ali *et al*., 2011b). The jejunal pieces were kept in petri dishes having Tyrode's solution, properly supplied with the Carbogen gas (95% O_2 and 5% CO_2) (Ali *et al*., 2011b). The tissues were then loaded in the organ bath containing 15ml of Tyrode's solution, already maintained on temperature 37±1^{0}C. Composition (mM) of Tyrode's solution was as: KCl 2.68,NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90,NaH_2PO_4 0.42,$CaCl_2$, 1.8 and glucose, 5.55 at a pH 7.4 (Ali *et al*., 2011b).The tissues were then subjected to stabilization for a period of 40-50 minutes. Test drug concentrations (0.01,0.1,1,10 and 15 µg/ml) of *moxifloxacin* and *gemifloxacin* were applied respectively in cumulative manner on 2 minutes duration between the dosing (Gilani *et al*., 1994). The experiments were repeated 5 times, otherwise mentioned (Faisal *et al*., 2018). The same procedure was repeated for effects of *nicorandil* as K Channels openers. The muscle relaxant effects of *nicorandil*, *moxifloxacin* and *gemifloxacin* on the low molar K^+ (20mM)-induced contractions were also tried in the presence of *glibenclamide*, a standard sulfonylurea that inhibits ATP sensitive potassium channels. Other students removed the part of interest for their studies. Rest of the bodies of animals were burnt in incinerator.

Identification of Mechanisms for Spasmolytic Activity

To identify the possible mechanisms for muscle relaxant activity observed on rabbits spontaneous jejunum preparations, test drugs (*moxifloxacin, gemifloxacin, nicorandil* and *verapamil)* were tested on rabbits' jejunal spontaneous rhythmic contractions, high (80mM)-molar KCl induced contractions; and on low (20mM)-molar KCl induced concentrations in the absence and presence of *glibenclamide* (3µM). The test drugs and standards were then added in a cumulative manner as reported by Vanrosum *et al*.,1996 protocols to elaborate the relaxant response mechanism (Gilani *et al*., 1994, Ali *et al*., 2016). The effects of *verapamil* were also tested on spontaneous, low molar K^+ (20mM)-induced contractions (With and without *glibenclamide*) and high (80mM)-molar KCl induced contractions The relaxant effect of the drug on the isolated tissues was expressed as % of control maximum response.

STATISTICAL ANALYSIS

Responses of the intestinal tissues to different test concentrations of test drugs were plotted as % of their respective response of control. Mean responses were plotted versus respective test concentrations. Respective EC_{50} values were calculated by using Graph Pad Prism version 6.

RESULTS

Effects of Moxifloxacin and Gemifloxacin on Rabbits' Jejunal Preparations

Effects of *moxifloxacin* and *gemifloxacin* on spontaneous rabbits' jejunal contractions, on low molar KCl (20mM)-induced contractions are, respectively, plotted in fig. 1 and fig. 2. Effects on high potassium induced contractions are also shown in fig. 1 and fig. 2. *Moxifloxacin* and *Gemifloxacin* relaxed the high (80mM) molar KCl induced contractions by 15% (*n*=5). Whereas their respective EC_{50} (for high molar K^+) were not determined as *moxifloxacin* and *gemifloxacin* could not relax the tissues to extent to calculate its $EC_{50.}$ When *moxifloxacin* and *gemifloxacin* were tested against the low molar KCl (20mM) induced contractions, relaxation of tissues was apparent on test concentration of 0.1µg/mL and onward as

shown in fig. 1 and fig. 2. Respective EC_{50} for *moxifloxacin* and *gemifloxacin* on spontaneous (without *glibenclamide*) contractions are 2.83±0.5µg/mL and 1.11±0.2µg/mL, respectively (n=5). While EC_{50} for *moxifloxacin* and *gemifloxacin* on spontaneous (in presence of *glibenclamide*) contractions could not calculate in presence of *glibenclamide* treated tissues as the tissues remained in sustained contractions (not relaxed).

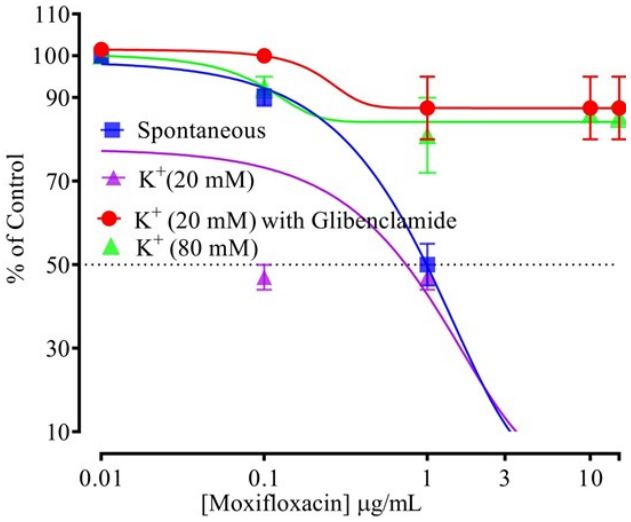

Fig. 1: Effects of *moxifloxacin* on rabbits' jejunal preparations on spontaneous (without *glibenclamide*), low molar (20mM)-KCl induced contractions, Low (20mM)-molar KCl induced contractions with *glibenclamide* (3 µM) and on high KCl (80 mM).
(All values are mean ±SD, *n*=5)

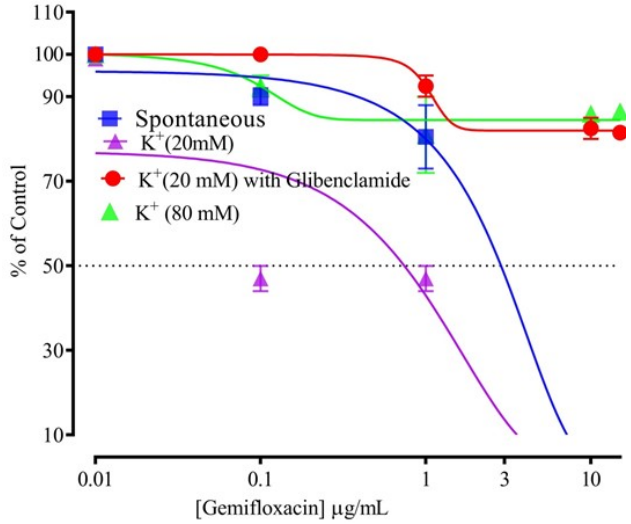

Fig. 2: Effects of *gemifloxacin* on rabbits' jejunal preparations on spontaneous (without *glibenclamide*), Low molar (20mM)-KCl induced contractions, Low (20mM) molar KCl induced contractions with *glibenclamide* (3µM) and on high KCl (80 mM).
(All values are mean ±SD, *n*=5).

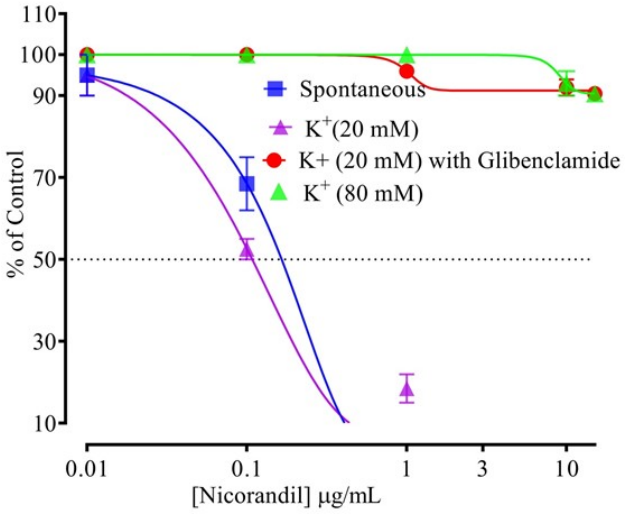

Fig. 3: Effects of *nicorandil* on rabbits' jejunal preparations on spontaneous (without *glibenclamide*), Low molar (20mM)-KCl induced contractions, Low (20mM)- molar KCl induced contractions with *glibenclamide* (3µM) and on high KCl (80mM).
(All values are mean ±SD, *n*=5)

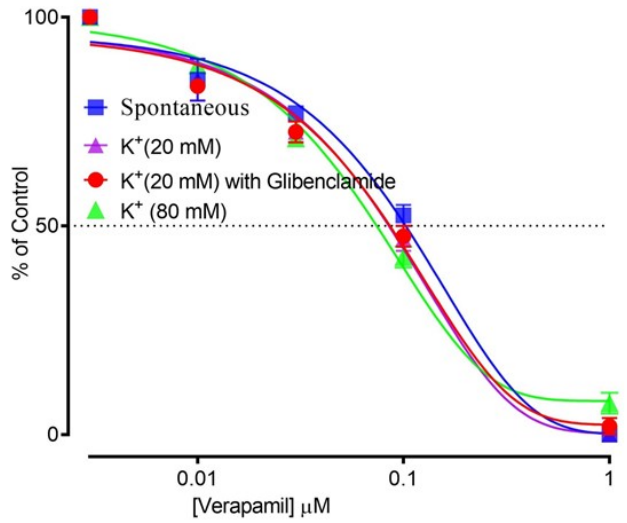

Fig. 4: Effects of *verapamil* effects of *moxifloxacin* on rabbits' jejunal preparations on spontaneous (without *glibenclamide*), Low molar (20mM)-KCl induced contractions, Low (20mM)-molar KCl induced contractions with *Glibenclamide* (3µM) and on high KCl (80mM). (All values are mean ± SD, *n*=3)

Similarly, *nicorandil* exhibited relaxing activity when checked on low K^+ (20 mM)-induced contractions (fig. 1 and fig. 2), which is a positive reference drug for potassium channel opening activity. The results showed complete relaxant effect on low 20mM K^+ - induced contractions in test concentration of 0.3µg/mL (fig. 1 and fig. 2). The EC_{50} for *nicorandil* was recorded as

0.16±0.01µg/mL (n=5). When *nicorandil* was checked on low molar KCl-induced contractions *glibenclamide* (3 µM), it relaxed low molar KCl-induced contractions only up to 32% (n=5) (fig. 3), therefore calculations of the EC_{50} were not possible. More, relaxant effects on high 80mM K^+-induced contractions on the rabbits' jejunal preparations was not up to 50% or more than 50% of control maximum to calculate EC_{50}. Thus, the observed weak relaxing effect on high K^+ (80mM) and *glibenclamide* sensitive relaxation of by low K^+ (20mM) induced rabbits jejunal contractions concludes the opening of K^+ ATPase channels. Concentration response curve for *verapamil* on spontaneous, on low potassium (with and without *glibenclamide*) and on high (80 mM) -molar KCl induced contractions are shown in fig. 4 (n=3). It is pertinent to mention that *Verapamil* relaxed all types of contractions with almost superimposable EC_{50} values.

DISCUSSION

Potassium channels openers have different pharmacological profile. They are vasodilators and antihypertensive drugs. As *moxifloxacin* and *gemifloxacin* demonstrated concentration dependent relaxation of isolated rabbits' jejunal preparations. So, the relaxant effect of *moxifloxacin* and *gemifloxacin* is evident. However, we do not know about the relaxing mechanism of *moxifloxacin* and *gemifloxacin* at this stage. Thus the relaxation effect was postulated either through inhibition of voltage gated calcium channels or K^+ channels activation (Ghayur *et al*., 2007), (Gilani *et al*., 2010). Hence, for further differentiation and confirmation for either involvement of calcium channels antagonist activity or K^+ channels activation activity (Gilani *et al*., 2006), the effects of *moxifloxacin* and *gemifloxacin* were tested in the presence of low K^+ and high K^+ induced contractions. As the calcium channels blockers have the tendency to equally inhibit both low and high KCl-induced contractions (Hamilton *et al*., 1986), (Gilani *et al*., 2005). Hence, we deduce that *verapamil* relaxed all types of contractions almost on equal EC_{50} values. Since, the high molar (80 mM) KCl-induced contractions are not relaxed by *moxifloxacin* and *gemifloxacin,* thus we can say that the relaxation effects are not mediated via calcium channels blocking activity as *moxifloxacin* and *gemifloxacin* only relaxed low KCl-induced contractions. This rule out, at this stage, the involvement of calcium channel antagonistic activity of *moxifloxacin* and *gemifloxacin* for the relaxation effect as weak inhibitory effect was observed on high molar KCl-induced contractions. Then for confirmation of involvement of potassium channels opening activity (Gilani *et al*., 2006), then the results for similar experimentations in the presence of *glibenclamide*, a specific blocker for ATP-dependent K^+ channels (Franck *et al*., 1994), (Shah *et al*., 2010) and in the presence of *nicorandil* helped in determination of relaxing mechanisms. This is because *nicorandil* is a known potassium channel opener drug which facilitates the entry of potassium into the cells and promotes efflux of sodium from the cells. So, the cells are not in state of depolarization and the muscles are relaxed. It is pertinent to mention that relaxant activity of *moxifloxacin* and *gemifloxacin* were prevented in the presence of *glibenclamide.* Glibenclamide is a known sodium potassium channel ATPase inhibitor, which don't promote entry of potassium into the cells and thus intracellular sodium level raises, which is followed by entry of calcium into the cells and keeps the tissues in sustained contracted form. As we observed the effect of *nicorandil,* a known ATP-sensitive K^+ channels opener (Escande *et al*., 1988), its activity was inhibited on ATP-sensitive potassium channels in the presence of *glibenclamide* on low K^+ induced contractions which implies that *moxifloxacin* and *gemifloxacin* has potassium channel opening activity which could be the reason for spasmolytic mechanism (Escande *et al*., 1988, Imtiaz *et al*., 2019, Siddiqui *et al*., 2020). It is interesting to note that *verapamil*, a standard calcium channels blocker, completely inhibited the contractions produced by low (20 mM)-molar and high (80mM)-molar KCl (Shah *et al*., 2010). Thus it is deduced that *moxifloxacin* and *Gemifloxacin* have relaxant effects possibly through the opening of ATP-sensitive K^+ channels that best describe its mechanism. This ATP-sensitive potassium channels opening activity of *Moxifloxacin* and *Gemifloxacin* could be a potential reason for relaxing activities that requires further work on this aspect.

CONCLUSION

The relaxant effect of *moxifloxacin* and *gemifloxacin* is predominantly mediated by activation of ATP-Sensitive potassium channels (K_{ATP}) opening activity that contributes to possible mechanism for spasmolytic activity on rabbits' jejunal preparations.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Pharmacy, Shaheed Benazir Bhutto University, Sheringal Dir Upper Khyber Pakhtunkhwa, Pakistan and Institute of Basic Medical Sciences, Khyber Medical University, Peshawar Khyber Pakhtunkhwa, Pakistan for making the resources available to carry out the research work.

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