ORIGINAL ARTICLE

# Influence of the Novel Anticancer Agents on the Activity of Outward Rectifier Potassium Currents in Human Prostate Cancer Cell Line - LNCaP

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## Abstract

**Background:** Phenolic compounds are ubiquitous in dietary fruits and vegetables and have been proposed to have antioxidant, antibacterial, anti-inflammatory, and anticancer effects. Voltage gated $K^+$ channels ($I_K$) contribute to various cellular mechanisms involved in the tumor cell progression. Therefore, the modulation of $I_K$ current may be a potential target for cancer therapy. In this study, we electrophysiologically characterized four phenolic compounds for their modulatory activities on $I_K$ currents. **Materials and Methods:** The whole cell configuration of the patch clamp recording technique was used to record the $I_K$ currents from human prostate cancer line (LNCaP). In addition, 200 µM of phenolic compounds (curcumin, resveratrol, rutin, and troxerutin) were externally perfused to the cells. **Results:** Obtained results provide evidence that resveratrol at 200 µM inhibited more than half of the $I_K$ current amplitude. On the other hand, curcumin, rutin, and troxerutin did not show any modulatory effect on $I_K$ current in LNCaP cells. **Conclusion:** This study demonstrates that not all the anticancer compounds are effective inhibitors of $I_K$ current, but only few of them. The $I_K$ current inhibitors might exhibit effective antimetastatic properties.

**Key words:** $I_K$ current, phenolic compounds, prostate cancer

## INTRODUCTION

Ion channels are involved in a large variety of physiological functions, including excitability, contraction, cell volume regulation, migration, cell cycle progression, and hormone secretion.[1] Most of these activities are directly or indirectly proposed to promote the complex process of cancer formation by various mechanisms.[2] Among different types of ion channels, abnormal expression of voltage gated $K^+$ channels ($I_K$) is reported in many cancer cells including prostate,[3] breast,[4] colon,[5] and glial.[6] In prostate cancer cells, the varying metastatic properties are distinguished by their ion channel characteristics.[7] However, it is well documented that $I_K$ channel blockers inhibit cell proliferation in prostate cancer cells.[8] Therefore, $I_K$ channel is one of the most interesting and promising therapeutic targets or prognostic marker in the fight against cancer, but selective $I_K$ channel modulators have hindered a critical verification for many years. In these aspects, investigation on the influence of novel compounds on the activity of $I_K$ channels seems to be new potential target for prostate cancer treatment.

Diets rich in phytochemicals have been associated with reduced risk in prostate cancer.[9] Plant-derived phenolic compounds are secondary metabolites in plants with a common aromatic ring with one or more hydroxyl groups. These phenolic compounds have been shown to exhibit antioxidant, anti-inflammatory, antithrombogenic, and anticancer activities. The anticancer activities of phenolic compounds have shown to be extended toward the inhibition of tumor initiation and progression by its complex molecular

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structure and chemical features, as well as its ability to interact with several signaling pathways.[10] Few studies emphasized that phenolic compounds affect a variety of ion channels and transporters. Especially curcumin and resveratrol exerts a modulatory effect on several ion channels, including voltage gated sodium and potassium channels.[11-13] However, to date, no studies investigated the modulatory effect of phenolic compounds curcumin, resveratrol, rutin, and troxerutin on voltage gated $K^+$ channel in human prostate cancer cell line LNCaP.

Therefore, in this study, we have made an attempt to investigate the modulatory effect of curcumin, resveratrol, rutin, and troxerutin on $I_K$ current in human prostate cancer cell line LNCaP.

## MATERIALS AND METHODS

### Reagents

Curcumin, resveratrol, rutin, and troxerutin were purchased from Sigma (St. Louis, MO, USA). The stock solutions were prepared in dimethyl sulfoxide (DMSO) and were stored in −20°C. All the drug solutions were freshly prepared from stock solutions before each set of experiments. The final concentration of DMSO was <0.1%.

### Cell culture

LNCaP cells were sourced from the National Center for Cell Science (Pune, India). LNCaP cells were cultured in RPMI-1640 medium (HiMedia Laboratories, India) supplemented with 10% fetal bovine serum and with 1% antibiotics (penicillin 100 IU/ml and streptomycin 100 mg/ml) in a humidified incubator at 37°C supplemented with 5% $CO_2$.

### Electrophysiology

Whole-cell patch recordings were performed on LNCaP cells. Recordings were made at room temperature. Pipettes were pulled from borosilicate glass capillaries with resistances of 2-3 MΩ when filled with internal solution. Currents were recorded using an Axon patch the Axopatch 200B patch-clamp amplifier (Axon Instruments, Sunnyvale, CA), Digidata 1322A (Axon Instruments), and PClamp software (version 6.0.3, Axon Instruments). The access resistance in our experiments was approximately 5-10 MΩ, and 40-60% series resistance compensation was achieved. Current records were acquired at 5 kHz and filtered at 2 kHz. The external solution used to record $K^+$ currents contained the following (in mM): NaCl 140, KCl 5, $MgCl_2$ 1, D-glucose 10, and HEPES 10, adjusted to pH 7.4 with 1 M NaOH. The internal solution contained the following (in mM): KCl 140, NaCl 5, $CaCl_2$ 2, $MgCl_2$ 1, D-glucose 10, and HEPES 10, adjusted to pH 7.2 with 1 M KOH. To evaluate the effect of phenolic compounds on the $I_K$ currents, the cells were held at a voltage of −80 mV, and membrane potential was stepped from −120 mV to +70 mV for 200 ms at 30 s intervals. All the recordings were performed with leak subtraction. The cell under investigation was continuously perfused with the external and drug solutions using the Octaflow (ALA instruments) perfusion system.

### Statistical analysis

The current-voltage curves were analyzed on ClampFit (9.2.1.9), IgorPro (5.04B), and Microsoft Excel 2012. All data values were calculated as a mean ± standard error of mean. Statistical significance of paired *t*-test and $P < 0.05$ was considered.

## RESULTS

### The effects of curcumin on $I_K$ current in LNCaP cells

We characterized four phenolic compounds for their modulatory activities on $I_K$ current in human prostate cancer cell line LNCaP. These compounds included flavonoids (curcumin, rutin, and troxerutin) and stilbenes (resveratrol). We first identified LNCaP cell lines that were expressing $I_K$ current. Then, we investigated the effect of curcumin on $I_K$ current in LNCaP cell line by depolarizing step pulse from −120 to +70 mV for 200 ms at 30 s was used to record the whole cell $I_K$ currents in LNCaP cells [Figure 1 - top left]. The representative current traces of before and after the exposure of 200 μM of curcumin are shown in Figure 1a. Current-voltage (I-V) curves for $I_K$ currents are constructed from the active currents [Figure 1b]. The I-V curve confirms that 200 μM of curcumin did not cause any effect on LNCaP cells.

### The effects of rutin on $I_K$ current in LNCaP cells

We have screened the effect of rutin on $I_K$ current in LNCaP cell line by depolarizing step pulse from −120 to +70 mV for 200 ms at 30 s was used to record the whole cell $I_K$ currents in LNCaP cells [Figure 2 - top left]. The representative current traces of before and after the exposure of 200 μM of rutin are shown in Figure 2a. The I-V relationship in the absence and presence of rutin is constructed [Figure 2b]. The I-V curve shows that 200 μM of rutin did not exert any effect on LNCaP cells.

### The effects of troxerutin on $I_K$ current in LNCaP cells

We further examined the effect of troxerutin on $I_K$ current in LNCaP cell line by depolarizing step pulse from −120 to +70 mV for 200 ms at 30 s was used to record the whole cell $I_K$ currents in LNCaP cells [Figure 3 - top left]. The

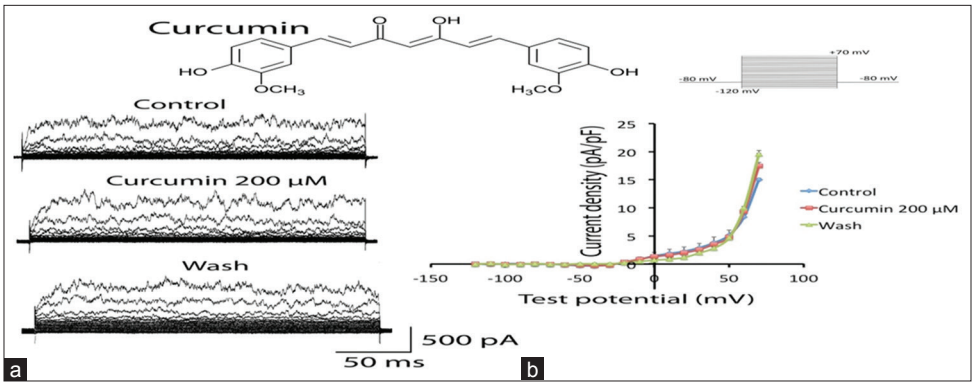


**Figure 1:** The effects of curcumin on $I_K$ current in LNCaP cells. (a) Representative traces of $I_K$ currents recorded in the presence and absence of curcumin 200 µM in LNCaP cells. (b) The current voltage (I-V) relationships of $I_K$ currents in the absence and presence of curcumin in LNCaP cells. Data are plotted as mean± standard error of mean ($n$ > 7)

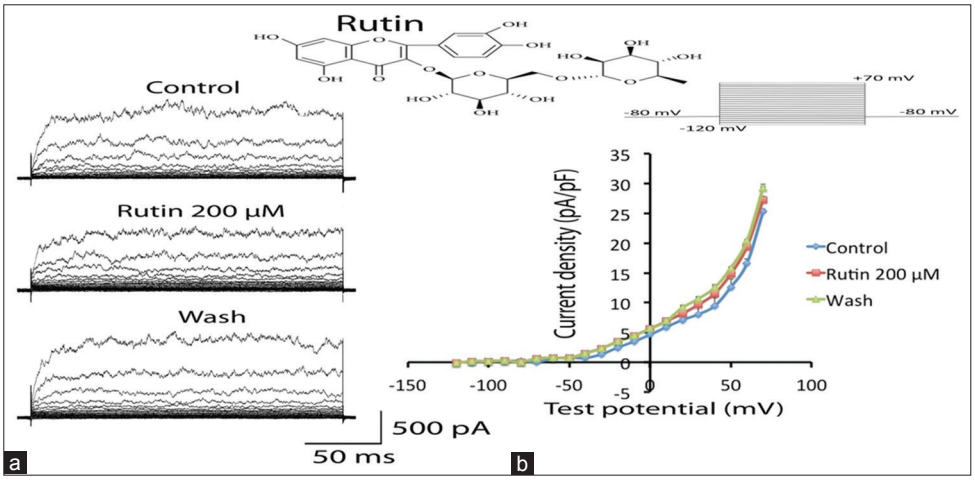


**Figure 2:** The effects of rutin on $I_K$ current in LNCaP cells. (a) Representative traces of $I_K$ currents recorded in the presence and absence of rutin 200 µM in LNCaP cells. (b) The current voltage (I-V) relationships of $I_K$ currents in the absence and presence of rutin in LNCaP cells. Data are plotted as mean ± standard error of mean ($n$ > 7)

representative current traces of before and after the exposure of 200 µM of troxerutin are shown in Figure 3a. Current voltage (I-V) curves for $I_K$ currents are plotted from the active currents [Figure 3b]. However, 200 µM of troxerutin also did not show any sign of $I_K$ current inhibition.

### The effects of resveratrol on $I_K$ current in LNCaP cells

All the three flavonoids (curcumin, rutin, and troxerutin) did not cause any inhibitory action on $I_K$ currents in LNCaP cells. Therefore, we further investigated the effect of resveratrol a stilbenes on $I_K$ current in LNCaP cell line by depolarizing step pulse from −120 to +70 mV for 200 ms at 30 s was used to record the whole cell $I_K$ currents in LNCaP cells [Figure 4 - top left]. The superimposed current traces of before and after the perfusion of 200 µM of resveratrol are shown in Figure 4a. Current-voltage (I-V) curves for $I_K$ currents are plotted from the active currents [Figure 4b]. The I-V curve confirms that 200 µM of resveratrol induce blockade in LNCaP cells. The peak current density plot also confirms that 200 µM resveratrol blocked $I_K$ currents in LNCaP cells [Figure 4c]. However, this effect was reversible immediate after the perfusion of external solution. Altogether, these results show that 200 µM of resveratrol blocked $I_K$ currents to more than half of the $I_K$ current in LNCaP cells.

## DISCUSSION

In this study, we investigated the effect of phenolic compounds (curcumin, resveratrol, rutin, and troxerutin) on the $I_K$ channel in human prostate cancer line-LNCaP. Our major findings were that among the four phenolic compounds, resveratrol inhibited $I_K$ current and the other three compounds curcumin, rutin, and troxerutin did not show any effect on $I_K$ current in LNCaP cells. These findings suggest that the $I_K$ current modulatory effect of any anticancer compounds firmly depends on their structural affinity with $I_K$ channel proteins. Flavonoids are the most important class of phenolic compounds. The anticancer properties of flavonoids have been reported in different types of cell lines. Despite a large number of flavonoids, we have selected curcumin, rutin, and troxerutin for investigating the $I_K$ channel activity

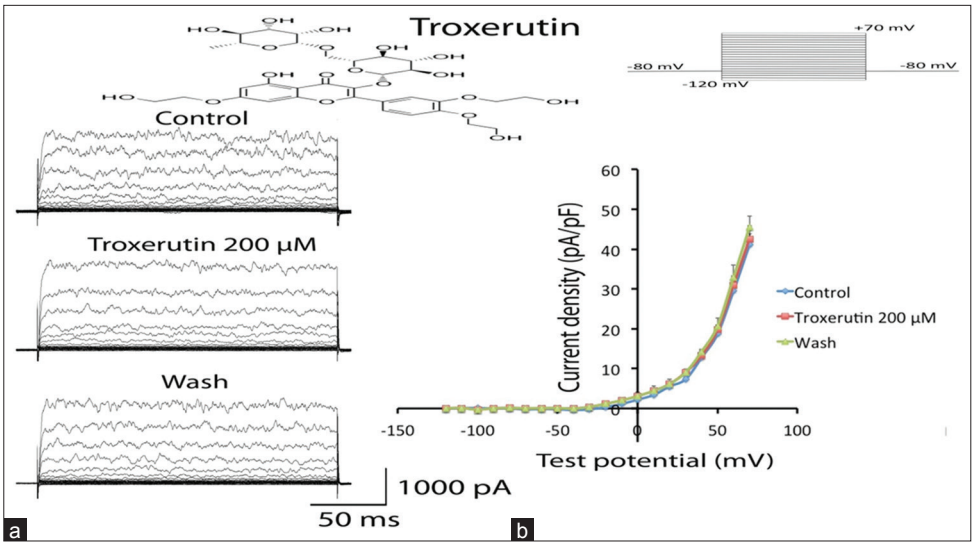


**Figure 3:** The effects of troxerutin on $I_K$ current in LNCaP cells. (a) Representative traces of $I_K$ currents recorded in the presence and absence of troxerutin 200 µM in LNCaP cells. (b) The current-voltage (I-V) relationships of $I_K$ currents in the absence and presence of troxerutin in LNCaP cells. Data are plotted as mean ± standard error of mean ($n > 7$)

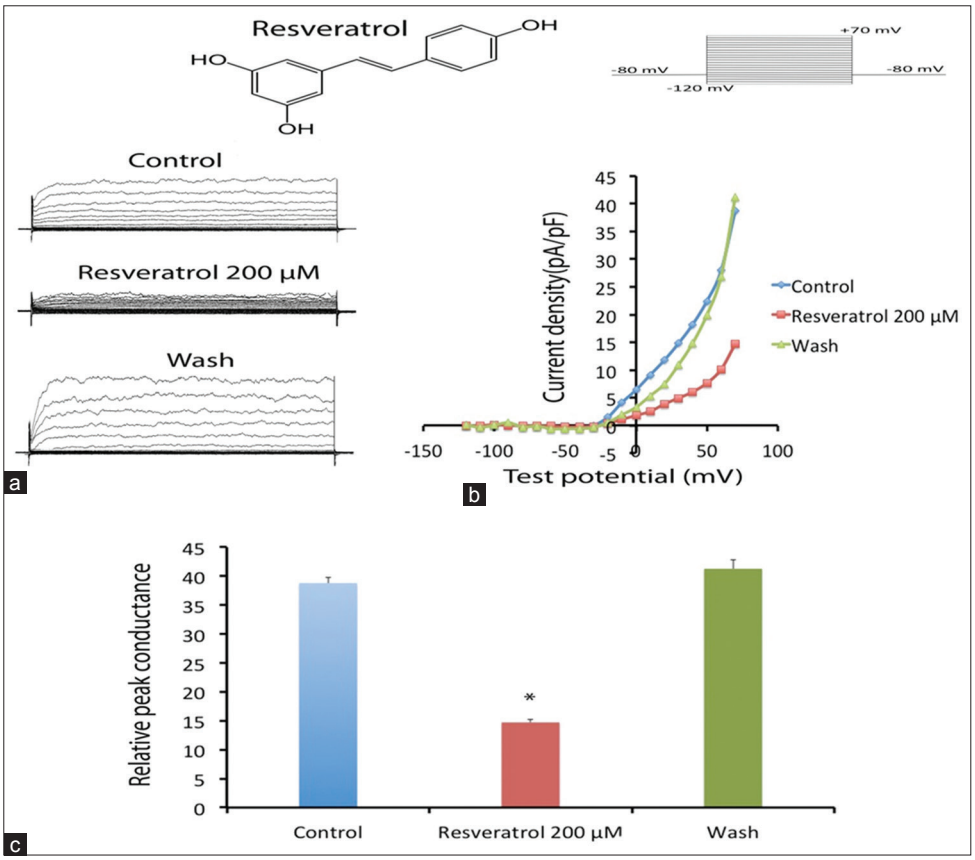


**Figure 4:** The effects of resveratrol on $I_K$ current in LNCaP cells. (a) Representative traces of $I_K$ currents recorded in the presence and absence of resveratrol 200 µM in LNCaP cells. (b) The current voltage (I-V) relationships of $I_K$ currents in the absence and presence of resveratrol in LNCaP cells. (c) Relative peak current density in the absence and presence of resveratrol in LNCaP cells. *$P < 0.05$ compared with the peak current amplitudes in the absence of resveratrol. Data are plotted as mean ± standard error of mean ($n > 7$)

in LNCaP cells. Among these three flavonoids, curcumin has shown to exert anticancer properties in many different types of cancer by modulating multiple signaling pathways. Based on its complex molecular structure, curcumin modulate several ion channels including voltage-gated potassium channels.[14] Curcumin blocked $I_K$ current in rabbit coronary

arterial smooth muscle cells,[15] inhibits Kv 1.3 in effector memory T cells,[16] and blocked Kv1.4 in bovine adrenal zona fasciculata cells.[12] However, our obtained results show that 200 μM of curcumin did not cause any modulatory effect on $I_K$ current in human prostate LNCaP cells. The other two flavonoids rutin and troxerutin exerted anticancer properties in several cancer cell lines through modulating multiple signaling pathways.[17] However, in prostate cancer cells rutin exhibited very low level of cell proliferation inhibition.[18] Our present study results reveal that rutin and troxerutin did not cause any changes in the $I_K$ current properties in human prostate LNCaP cells. Altogether these results suggest that the $I_K$ current inhibition depends on the interaction between the $I_K$ channel protein and the chemical structure of the compound.

Resveratrol (3,4,5-trihydroxystillbene), a natural polyphenolic compound commonly present in natural products such as grapes, berries, peanuts, and soybeans has been suggested to treat variety of cancer types including prostate cancer.[19] It has been reported to modulate several signaling pathways to control the growth of cancer cells.[20] In addition, resveratrol has shown to attenuate several voltage gated ion channels in several cell lines. Mainly resveratrol inhibited voltage gated sodium current in rat prostate cancer cell line (MAT-LyLu)[11] and Kv1.3 in human lymphocytes.[21] Our obtained result shows that perfusion of 200 μM of resveratrol inhibited $I_K$ current in LNCaP cells. When compared to control, the I-V curve shows that 200 μM of resveratrol inhibited more than half of the $I_K$ current. The peak current also shows significant inhibition of $I_K$ current in LNCaP cells. However, this inhibitory effect was reversible immediate after the perfusion of external solution. These results suggest that resveratrol effectively inhibit the $I_K$ current in human prostate cancer cell line LNCaP.

## CONCLUSION

The results provide evidence that not all the tested phenolic compounds applied at a concentration of 200 μM appeared to be an inhibitor of $I_K$ current in LNCaP cells. This confirms that not all the anticancer compounds have the ability to inhibit or interact with $I_K$ channel but only a few of them. Our obtained results suggest that, when compared to flavonoids (curcumin, rutin, and troxerutin), stilbenes (resveratrol) are effective inhibitor of $I_K$ current in human prostate cancer cell line LNCaP. However, we cannot ignore that these tested compounds are incompetent in blocking ion channels. The tested compounds may inhibit $I_K$ current at higher dosages. Further, studies are required on higher dosages of these compounds and other types of cell lines.

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