Tirzepatide Suppresses NF- κB Gene Expression in Colorectal Cancer Cells In Vitro

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Abstract

Background: Overweight and obesity prevalence have surged globally between 1990 and 2021. One billion males and 1.11 billion females are either overweight or obese. **Aim of the study:** This study investigates the in vitro effect of the dual glucose-dependent insulinotropic polypeptide and Glucagon-like peptide-1 GIP/GLP-1 receptor agonist Tirzepatide on the gene expression of nuclear factor kappa B (NF-κB), a transcription factor critically involved in inflammation and carcinogenesis. Given the potential link between incretin-based therapies and cancer risk, we hypothesize that Tirzepatide might upregulate NF-κB.
Methods: Colorectal SW48 cells were treated with three concentrations of Tirzepatide (0.05, 0.5, and 5 µg/µl) for 24 hours. RNA was extracted, cDNA was synthesized, and gene expression was quantified using qPCR.
Results: Contrary to the initial hypothesis, results demonstrated a significant, dose-dependent downregulation of NF-κB gene expression. The most substantial suppression (0.03-fold change) was observed at the 0.5 µg/µl concentration, suggesting a non-linear, optimal dose-response relationship. These findings indicate that Tirzepatide exerts a direct anti-inflammatory effect by inhibiting NF-κB signaling in a controlled cellular environment. The observed suppression contrasts with clinical concerns, suggesting that any associated cancer risk may arise from secondary, systemic pathological factors rather than a direct oncogenic effect of the drug.
Conclusion: This study highlights Tirzepatide's potential cytoprotective role through NF-κB pathway inhibition.
Keywords: Colorectal Cancer, in vitro, Tirzepatide, obesity, DM

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Introduction

Overweight and obesity prevalence is in surge globally between 1990 and 2021. One billion male and 1.11 billion female are either overweight or obese and some countries in the middle east have obesity reaches 80% (Ng et al., 2025). Various interventions are developed to combat obesity including diet, medicines such as liraglutide Glucagon-like peptide-1 (GLP-1) or intragastric ballons and bariatric surgery [6]. Tirzepatide, a first-in-class “Twincretin” that is approved by U.S Food and Drug Administration FDA on May 13th of 2022, The drug has dual stimulation action in (GIP) pathway and GLP-1R and improves blood sugar control in type2 diabetics (T2D). The pharmaceutical company Eli Lilly and Co.® developed the medication, which sold under the trade name "Mounjaro™."[5].

The activation process triggers the expression of insulin, insulin-like growth factors 1 and 2 (IGF 1 and 2) and insulin-like growth factor binding protein 3 IGF-BP3, and initiates the transcription of the insulin gene. These molecules then bind to insulin or IGF receptors on target cells to activate crucial signaling [7]. Abnormal activation of these pathways, may lead to pancreatitis could be transformed to pancreatic cancer and could also cause other types of cancers including breast cancer, liver and colon [8], [9] . Cancers could be consequently developed due to

several pathways, promoting cellular growth and proliferation, abnormal cancer metabolism, and the prevention of cell death, invasion, and metastasis in these tissues, all of which contribute to the advancement of cancers [10].

The essential nuclear factor kappa B (NF-kB) family of transcription factors work as stressors in the cellular environment. NF-kB controls the expression of vital regulatory genes involved in immunity, inflammation, cell division, and death. The cytoplasmic NF-kB protein is activated by a variety of biological events. In a complex signaling pathway that involves molecular interactions with adaptor proteins and phosphorylation and ubiquitinase enzymes, both the canonical and non-canonical pathways, activate NF-kB. This in turn regulates gene expression and promotes NF-kB translocation within the nucleus [11].

NF-κB activation is associated with tumor cell resistance to chemotherapy and radiation, as well as the regulation of the apoptotic pathway, cell proliferation, differentiation, migration, and angiogenesis. Breast cancer, ovarian cancer, prostate cancer, gastric carcinoma, and colorectal cancer are among the cancers for which NF-κB is known to have a significant role. Targeting NF-κB could result in new treatment strategies and preventative measures for human malignancies [12].

Numerous studies demonstrate that different cancer cell lines have activated NF-κB signaling pathways. Stimulation of NF-κB signaling pathway significantly contributes to carcinogenesis by controlling the downstream NF-κB gene products in colorectal cancer . When NF-kB and its dependent genes are down-regulated, cancer cells exhibit increased apoptotic cell death and drug sensitivity while also experiencing decreased cell proliferation, inflammation, metastasis, and angiogenesis [13] (Soleimani et al., 2020).

So, aim of this study is to analyze Tirzepatide mechanism of action in vitro by assessing gene expression of NF-kB by treating cells then comparing it with the control treated samples.

Methodology

In Vitro Analysis

SW48 Cell line (Colorectal cells), that is utilized in this study were obtained from the cell culture techniques company Rawafid Aleloom, located in Hilla, Iraq. Rawafid Aleloom supplied to the central lab/ College of Medicine/ University of Basrah, Basrah, Iraq. Once the cell batch arrived to the laboratory, the cells are refreshed with fresh 10% FCS supplemented DMEM. The confluent cell mono-layer is sub-cultured by trypsinsation. Cells are plated out into 24 well plate at 1 ×105 cells / ml. Plated cells are left to grow for 24 hours and then treated with 0.05, 0.5 and 5 µg / µl concentrations of mounjaro as triplicates for each. The fourth line is left as control which is treated with DMEM- Serum free media. Samples are harvested 24 hours later mechanically using sterile tips and transferred to sterile Eppendorf tubes and are kept at -20 C. The harvest is used for downstream analysis, RNA extraction, cDNA synthesis and gene expression analysis of the concerned gene.

RNA Extraction and cDNA synthesis

RNA samples from treated and control samples of (SW48) are isolated following the instruction of Solarbio, Cat No: R1200 following steps. In order to convert RNA to cDNA, the procedure is conducted according to Universal RT-PCR Kit (M-MLV, free Taq polymerase) from Salorbio, Cat No: RP1105and the mixture is stored at -20°C immediately for downstream analysis.

Gene Expression Quantification by Real-Time PCR

The Real Time PCR analysis is conducted a kit from SolGent Co., Ltd. (catalog number 34014, Korea) table 1 & 2.

Statistical Analysis

The study utilized unpaired t-tests (excel software) to assess the disparities among the experimental groups. It is determined that $p < 0.05$ is statistically significant.

Results and discussion

Gene Expression Analysis

RNA concentration and quality of all samples are measured using nanodrop (Thermos Scientific-UK). All samples' concentrations are adjusted to the lowest concentration. The qPCR reaction is initiated at equal concentrations to eliminate the bias in relative and fold change analysis. Melting curve analysis is added to the

qPCR program to assess the specificity of the primers and to test of the sybr (intercalating dye) produced a single, specific product using the Rotor gene-Q software. Each amplification curve is considered when the amplification curve crossed the threshold value. The amplification plot is visualized with the two axes in a lineal scale or what is so called Lineal Amplification Plot (LAP). The none-template control NTC didn't give any signal and consistently

remained below the threshold. Distinctive mountain peaks of NF-kB was detected. While the fluorescence plots are typically detected for all genes using specific primers. Another clean specificity and amplification curve are detected for β-actin as the housekeeping gene

NF-kB gene expression

Altered expression of NF-κB may result in novel treatments and prevent human cancers. Using NF-kB -specific primers, the cDNA templates are combined to a qPCR SYBR green master mix in order to estimate the gene expression of NF-kB in samples. NF-kB downregulated gene expression is reported in all the treated samples. Data are standardized to β-actin and calculated using ΔΔ analysis figure1. positive value is parallel to the control sample, which is normalized to 1, In order to mark the fold shift value in the treated samples the decrease is at a rate of 0.6, 0. 9 and 0.3 figure compared to the control figure 1 & 2.

Figure 1: A SYBR green master mix for the qPCR relative expression test, adjusted to β-actin, is used to reverse transcribe the extracted RNA after the expression level of NKFB is assessed in both positive and negative samples (P=0.7).

Figure 2: Fold change gene expression analysis. Value of the control samples are adjusted to 1 and all the treated samples are compared to that 1 to extract the fold changes values.

Discussion

This study shown that Tirzepatide dramatically downregulated NF-κB gene expression in a dose-dependent manner in the examined cell line. In comparison to the control group (established at 1.0), administration of Tirzepatide at 5 μg reduced the expression NF-κB to 0.29, at 0.5 μg to 0.03, while the lower dosage of 0.05 μg led to a more moderate decrease to 0.67, indicating a potential non-linear dose–response relationship. The findings indicate that Tirzepatide inhibits NF-κB signaling, especially at moderate treatment, underscoring its potential as an anti-inflammatory drugs.

Interestingly, these findings came in contrast to the initial hypothesis. NF-κB, a pivotal transcription factor in inflammation and tumorigenesis, this gene is anticipated to be activated upon exposure to (1-4). Since NF-κB promotes inflammation, cell proliferation, and resistance to apoptosis, such negative effects would normally be linked to an overexpression of the protein. Our findings showed a considerable decrease of NF-κB expression, which is contrary to what is expected (5, 6).

Our conclusion comes in consistent with increasing evidence of recent data suggesting that tirzepatide has cytoprotective and anti-inflammatory effects in some situations. For instance, Tirzepatide inhibited\s the TLR4/NF-κB/NLRP3 pathway to reduce ventricular remodeling and dysfunction in a mouse model of lipopolysaccharide (LPS)-induced heart damage(7). Similarly, via inhibiting PI3K/p-Akt/GSK3β/NF-κB p65 signaling, tirzepatide decreased renal and CNS inflammation in a model of colistin-induced nephrotoxicity(8).

Tirzepatide has also been demonstrated to reduce inflammation and oxidative stress in models of metabolic diseases. By altering the IL-17 signaling pathway, it decreased NF-κB activity in diabetic nephropathy(9). In high-fat diet-induced cognitive decline, it improved memory and reduced neuroinflammation by targeting the SIRT3-NLRP3 axis, indirectly reducing NF-κB activation (10). Furthermore, by inhibiting aberrant insulin resistance and NF-κB signaling in the brain, tirzepatide enhanced spatial learning in diabetic rats(11).

Importantly, the disparities between acute exposure in controlled cell culture experiments and chronic in vivo exposure in disease-prone experimental animals may account for the discrepancy between expected and observed outcomes. Instead of direct Tirzepatide signaling, NF-κB activation most likely results from tissue remodelling, oxidative stress, or carcinogenic mechanisms in clinical and preclinical studies of thyroid or pancreatic problems.

The lowest concentration of the substance (0.05 μg) resulted in only a partial suppression (0.67), suggesting that minimal receptor engagement or incomplete pathway activation does not fully inhibit NF-κB. This finding is consistent with the hypothesis that insufficient agonism of GLP-1R and GIPR may limit downstream anti-inflammatory effects(12). The intermediate concentration (0.5 μg) exhibited the most significant reduction (0.03), indicating an optimal signaling threshold where Tirzepatide's combined GLP-1R/GIPR activation maximally suppresses NF-κB–mediated transcription. The existence of biphasic or U-shaped dose–response curves has been documented in the case of incretin mimetics. The moderate doses of these mimetics have been shown to yield stronger protective effects in comparison to very high doses. This phenomenon can be attributed to receptor de-sensitization or feedback signaling loops(11).

The highest concentration (5 μg) led to a substantial (0.29) reduction in NF- κB expression, such relative

attenuation could be indicative of receptor downregulation, intracellular signaling saturation, or compensatory pro-inflammatory mechanisms activated at supraphysiological doses. Studies have demonstrated that prolonged or high-dose GLP-1 analogue exposure can unexpectedly result in the activation of stress responses and low-grade inflammation. Therefore, it can be concluded that Tirzepatide's anti-inflammatory efficacy follows a non-linear dose relationship, with maximal benefit being achieved at intermediate levels.

Linking Tirzepatide to pancreatic inflammation and thyroid tumourigenesis and its observed suppression of NF-κB in this study highlights the complexity of incretin pharmacology. In pathological tissues that are predisposed to oncogenesis or chronic inflammation, NF-κB may be subject to secondary upregulation by factors such as oxidative stress, tumourigenic signaling, or metabolic dysregulation. This upregulation is believed to occur as a consequence of these factors rather than as a direct result of Tirzepatide action (14-16). Conversely, in our in vitro model, which is devoid of these confounding systemic factors, Tirzepatide primarily exerts its independent anti-inflammatory role.

Oxidative stress maintains NF-κB activation via cytokine release (TNF-α, IL-1, IL-17) and reactive oxygen species (ROS) production, therefore fostering genomic instability and cancer. Furthermore, NF-κB has been demonstrated to alter cellular metabolism by promoting glycolysis and inhibiting oxidative phosphorylation in the absence of p53, thereby associating metabolic dysregulation with persistent NF-κB activity. Ultimately, oncogenic mutations like Kras and p53 deficiency collaborate to sustain NF-κB signaling and shield tumour cells from apoptosis, reinforcing the idea that NF-κB upregulation in pathological tissues frequently occurs as a secondary event induced by tumorigenic signaling rather than as a direct consequence of drug action(17). Conversely, in our in vitro cell line model away from all these confounding systemic variables, Tirzepatide displayed its principal anti-inflammatory function.

Conclusion

The observed suppression contrasts with clinical concerns, suggesting that any associated cancer risk may arise from secondary, systemic pathological factors rather than a direct oncogenic effect of the drug. This study highlights Tirzepatide's potential cytoprotective role through NF-κB pathway inhibition.

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Author's Contributions

Author contribution: NA, conducted the research experiments and drafted the first draft, ZA critically reviewed the paper and study supervision, JA critically reviewed the paper and study supervision.

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Table1: Components of q- PCR reaction in real time.

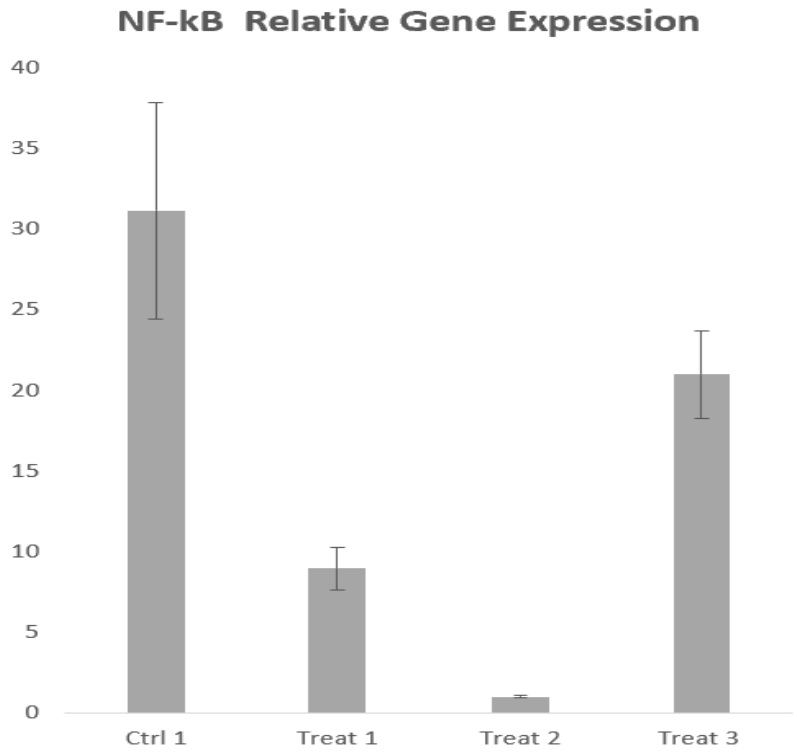
Materials	Size
solGent Co., Ltd	10 µl
Forward of primers	100 pmol
Revers of primers	100 pmo;
Nuclease free water	6 µl
Template cDNA	15 ng
Final size	20 µl

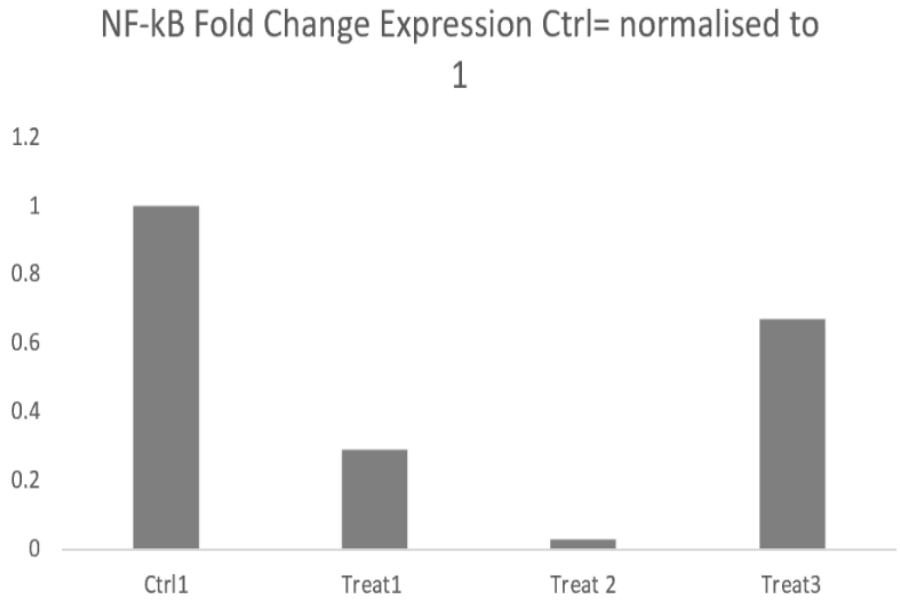
Table 2: Real Time PCR conditions

No.	Steps	TM	Time	No. of cycles
I	Denaturation I	94°C	3 min	1
II	Denaturation II	94°C	30 sec	40
III	Annealing	60°C	30 sec	
IV	Extension I	72°C	1 min	
V	Extension II	72°C	5 min	1

Table: 3: qPCR primers used in this study

NF KB	F	5'– TTACGGGAGATG TGAAGATG -3'	Macrogen	Korea	Janbandhu et al.,2010)
	R	5'– GAGTTATAGGCC TCAGGGTACTCC AT -3'			


NF-kB Relative Gene Expression
40
35
30
25
20
15
10
5
0
Ctrl 1
Treat 1
Treat 2
Treat 3


NF-kB Fold Change Expression Ctrl= normalised to 1
1.2
1
0.8
0.6
0.4
0.2
0
Ctrl1
Treat1
Treat 2
Treat3