RESEARCH PAPER

Diosmin Hampers NDEA-Induced Changes in Inflammatory Signalling Molecules in Liver Cancer Induced Rats

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ABSTRACT

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide and is closely associated with chronic inflammation and dysregulation of inflammatory signalling pathways. The present study aimed to evaluate the chemopreventive efficacy of diosmin against N-nitrosodiethylamine (NDEA)-induced hepatocarcinogenesis by assessing key inflammatory signalling molecules in experimental rats.

Healthy adult male Wistar rats were divided into four groups: control, NDEA-induced hepatocellular carcinoma, diosmin-treated cancer group, and diosmin-alone group. Diosmin was administered orally at a dose of 200 mg/kg body weight for 28 days. The gene expression levels of inflammatory mediators, including inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), were analyzed using amplification-based techniques.

The results revealed a significant upregulation of inflammatory gene expression in NDEA-induced rats, whereas diosmin treatment markedly attenuated these changes. The cycle threshold values ranged between 20–30 cycles, confirming active transcriptional regulation. Diosmin exhibited potent anti-inflammatory effects by modulating cytokine expression and suppressing inflammatory signalling pathways.

In conclusion, diosmin demonstrates promising chemopreventive potential against hepatocarcinogenesis by targeting key inflammatory mediators, suggesting its potential role in liver cancer prevention and therapy.

Keywords: Diosmin, Hepatocellular carcinoma, NDEA, Inflammatory signalling, IKKβ, IL-1β, TNF-α, Chemoprevention, Cytokines, Liver cancer.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies globally and represents a major public health concern due to its high morbidity and mortality rates [1]. The development of HCC is closely linked to chronic inflammation, oxidative stress, and persistent liver injury, which collectively contribute to tumor initiation and progression [2].

Experimental models using chemical carcinogens such as N-nitrosodiethylamine (NDEA) are widely employed to study hepatocarcinogenesis, as they closely mimic the pathological and molecular alterations observed in human liver cancer [3]. NDEA induces DNA damage, oxidative stress, and activation of inflammatory signalling pathways, thereby promoting tumor formation.

Liver regeneration and repair involve a complex interplay of cytokines, growth factors, and signalling molecules that regulate cellular proliferation and immune responses [4]. Among these, inflammatory mediators such as IKKβ, IL-1β, and TNF-α play a pivotal role in modulating immune responses and maintaining inflammatory homeostasis. However, persistent activation of these mediators contributes to chronic inflammation and tumor development [5].

The nuclear factor-kappa B (NF-κB) signalling pathway, regulated by IKKβ, is a key mediator of inflammation and cancer progression. Cytokines such as IL-1β and TNF-α further amplify inflammatory responses, creating a tumor-promoting microenvironment [6].

Recent research has focused on identifying natural compounds with anti-inflammatory and anticancer properties that can modulate these pathways. Diosmin, a naturally occurring flavonoid, has gained attention due to its antioxidant, anti-inflammatory, and chemopreventive properties [7]. It has been shown to inhibit inflammatory cytokines and reduce oxidative

stress, thereby potentially preventing cancer progression.

The present study aims to evaluate the effect of diosmin on inflammatory signalling molecules in NDEA-induced hepatocellular carcinoma in rats, with a focus on its role in regulating key cytokines involved in inflammation and tumor development.

AIM

To evaluate the effect of diosmin on inflammatory signalling molecules in NDEA-induced hepatocellular carcinoma in experimental rats.

OBJECTIVES

- To induce hepatocellular carcinoma using N-nitrosodiethylamine (NDEA) in Wistar rats
- To analyze the expression of inflammatory signalling molecules (IKKβ, IL-1β, TNF-α)
- To evaluate the chemopreventive efficacy of diosmin
- To compare gene expression levels among control, cancer-induced, and treated groups
- To understand the role of inflammatory pathways in hepatocarcinogenesis

MATERIALS AND METHODS

Healthy adult male albino Wistar rats weighing 180–200 g were used in this study, following institutional ethical guidelines. The study was approved by the Institutional Animal Ethical Committee.

The animals were divided into four groups:

- **Group I:** Control
- **Group II:** NDEA-induced hepatocellular carcinoma
- **Group III:** NDEA-induced rats treated with diosmin (200 mg/kg/day orally for 28 days)
- **Group IV:** Control rats treated with diosmin alone

Gene expression analysis of inflammatory markers was performed using amplification-based molecular techniques.

RESULTS

Gene Expression Analysis of Inflammatory Markers

The relative mRNA expression levels of key inflammatory signalling molecules—IKKβ, IL-1β, and TNF-α—were analyzed across all experimental groups using quantitative amplification-based molecular techniques.

A **significant upregulation** of all three inflammatory markers was observed in the NDEA-induced hepatocellular carcinoma (HCC) group (Group II) when compared with the control group (Group I). This elevation indicates a strong activation of inflammatory pathways associated with hepatocarcinogenesis.

- **IKKβ expression** was markedly increased in the NDEA group, reflecting activation of the NF-κB signalling cascade.
- **IL-1β and TNF-α levels** were also significantly elevated, indicating an enhanced pro-inflammatory cytokine response.

In contrast, the control group exhibited **baseline physiological expression levels**, confirming normal hepatic homeostasis.

Effect of Diosmin on Gene Expression

Treatment with Diosmin (Group III) resulted in a **significant downregulation** of all studied inflammatory markers compared to the untreated NDEA-induced group.

- A substantial reduction in **IKKβ expression** suggests inhibition of NF-κB pathway activation.
- **IL-1β and TNF-α levels** were notably decreased, indicating suppression of inflammatory cytokine production.

The group treated with diosmin alone (Group IV) showed no significant alterations in gene expression compared to the control, suggesting that diosmin does not adversely affect normal physiological processes.

Amplification and Quantitative Analysis

Quantitative PCR amplification curves demonstrated typical sigmoid patterns, confirming the reliability of gene expression measurements.

- The **cycle threshold (Ct) values** ranged between 20–30 cycles across experimental groups.
- Lower Ct values in the NDEA group indicated **higher gene expression levels**, whereas increased Ct values in diosmin-treated groups reflected **reduced transcriptional activity**.

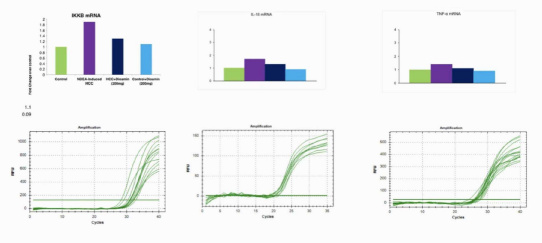
These findings validate the quantitative differences observed in inflammatory gene expression among the groups.

Comparative Analysis Between Groups

A comparative assessment revealed:

- **Group II (NDEA-induced)**: Highest expression of inflammatory markers
- **Group III (NDEA + Diosmin)**: Significant reduction in expression
- **Group I (Control)**: Baseline expression
- **Group IV (Diosmin alone)**: Comparable to control

Overall, diosmin treatment effectively reversed NDEA-induced inflammatory gene upregulation.



DISCUSSION

Inflammation and Hepatocarcinogenesis

Hepatocellular carcinoma is strongly associated with chronic inflammation, which plays a central role in tumor initiation, promotion, and progression. In the present study, NDEA administration resulted in a marked increase in inflammatory mediators, confirming its ability to induce hepatic carcinogenesis through inflammatory mechanisms.

The observed upregulation of **IKKβ, IL-1β, and TNF-α** highlights the activation of key signalling pathways involved in liver tumor development.

- **IKKβ** is a critical regulator of the NF-κB pathway, which controls the transcription of genes involved in inflammation, cell survival, and proliferation.
- **IL-1β and TNF-α** are potent pro-inflammatory cytokines that contribute to a tumor-promoting microenvironment by enhancing oxidative stress, DNA damage, and cellular proliferation.

These findings are consistent with previous studies demonstrating that persistent inflammatory signalling drives hepatocellular carcinoma progression.

Role of NF-κB Signalling Pathway

The activation of IKKβ observed in the NDEA group suggests enhanced NF-κB pathway activity. NF-κB is known to regulate genes involved in:

- Anti-apoptotic mechanisms
- Cell proliferation
- Angiogenesis
- Immune modulation

Sustained activation of this pathway promotes tumor survival and resistance to apoptosis, thereby facilitating cancer progression. The elevated expression of downstream cytokines such as TNF-α and IL-1β further amplifies this inflammatory cascade.

Anti-Inflammatory and Chemopreventive Effects of Diosmin

Treatment with diosmin resulted in a significant reduction in the expression of inflammatory markers, indicating its potent anti-inflammatory activity.

The observed downregulation of IKKβ suggests that diosmin may inhibit NF-κB activation, thereby interrupting the inflammatory signalling cascade at an upstream level. This effect is further supported by the decreased levels of IL-1β and TNF-α, which are downstream targets of NF-κB.

The chemopreventive effects of diosmin can be attributed to multiple mechanisms:

- **Inhibition of inflammatory signalling pathways**
- **Reduction of oxidative stress**
- **Modulation of cytokine production**
- **Prevention of DNA damage and cellular transformation**

These properties collectively contribute to its protective role against hepatocarcinogenesis.

Protective Role Against Oxidative Stress

NDEA-induced liver injury is known to generate reactive oxygen species (ROS), leading to oxidative stress and cellular damage. Diosmin, being a flavonoid, possesses strong antioxidant properties that help neutralize ROS and protect hepatocytes from oxidative injury.

By reducing oxidative stress, diosmin indirectly suppresses inflammation and prevents activation of pro-inflammatory signalling pathways.

Clinical and Therapeutic Implications

The findings of this study highlight the potential of diosmin as a natural therapeutic agent for the prevention and management of liver cancer. Targeting inflammatory pathways represents a promising strategy in cancer therapy, as chronic inflammation is a key driver of tumor progression.

Compared to conventional chemotherapeutic agents, natural compounds like diosmin offer several advantages:

- Lower toxicity
- Better safety profile
- Multifaceted mechanisms of action

Thus, diosmin may serve as an effective adjunct or alternative in cancer prevention strategies.

Limitations and Future Perspectives

Although the study demonstrates promising results, certain limitations should be acknowledged:

- Lack of protein-level validation (e.g., Western blot or ELISA)
- Absence of long-term survival studies
- Limited mechanistic exploration of signalling pathways

Future studies should focus on:

- Detailed molecular pathway analysis
- In vivo long-term efficacy studies
- Clinical trials to validate translational potential

CONCLUSION

The present study demonstrates that diosmin effectively attenuates NDEA-induced alterations in inflammatory signalling molecules in hepatocellular

carcinoma. The significant upregulation of IKKβ, IL-1β, and TNF-α in cancer-induced rats confirms the role of inflammation in hepatocarcinogenesis.

Diosmin treatment markedly reduced the expression of these inflammatory markers, highlighting its anti-inflammatory and chemopreventive properties. These findings suggest that diosmin may serve as a potential therapeutic agent for the prevention and management of liver cancer.

Further studies are required to elucidate its molecular mechanisms and validate its clinical applications.

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