Int. J. Morphol.,
42(6):1628-1637, 2024.

Synergistic Effect of Caloric Restriction and Cyclooxygenase-2 Inhibitor "Celecoxib" Against IGFs/COX-2 Mediating Chemically Induced Hepatocellular Carcinoma in Rats

Efecto Sinérgico de la Restricción Calórica y el Inhibidor de la Ciclooxigenasa-2 "Celecoxib" Contra los IGF/COX-2 que Median el Carcinoma Hepatocelular Inducido Químicamente en Ratas

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SHAMSELDEEN, A. M.; EL HAMZAWY, M.; ABOULHODA, B. E.; RASHED, L; ALGHAMDI, M. & HASSAN, F. E. Synergistic effect of caloric restriction and cyclooxygenase-2 inhibitor "Celecoxib" against IGFs/COX-2 mediating chemically induced hepatocellular carcinoma in rats. *Int. J. Morphol., 42(6)*:1628-1637, 2024.

SUMMARY: Caloric restriction (CR) alongside its ability to increase longevity, exerts inhibitory effects against various tumors. The purpose of this current research was to investigate the conceivable implications of CR, either independently or in conjunction with a cyclooxygenase 2 inhibitor (Celecoxib), on the pathophysiology of induced hepatocellular carcinoma (HCC). Forty adult male Wistar rats were allocated into four groups: Control, HCC; induced by injecting diethylnitrosamine intraperitoneally, then subsequently given a weekly subcutaneous injection of carbon tetrachloride (CCl_4) once a week for 6 consecutive weeks, CR group, and CR-Celecoxib group. The results revealed enhanced serum insulin growth factor-I (IGF-I) and- II (IGF-II) levels, along with a significant increase in COX-2 and IL-6 gene expression as well as acceleration of pathological changes, glycogen depletion, enhanced fibrosis, and decreased caspase 3 expression in the liver. However, both IGF-I, II, and alpha-fetoprotein (AFP) were significantly decreased in the CR group co-treated with Celecoxib which was positively reflected on the liver architecture. We concluded that combined CR and COX-2 inhibition interferes with the pathogenesis of HCC.

KEY WORDS: Hepatocellular carcinoma; COX2; IGF; Celecoxib; Caloric restriction.

INTRODUCTION

Hepatocellular inflammation and proliferation are hallmarks of the multi-tiered hepatocarcinogenesis process. It has been speculated that erroneous expression of different growth factors, oncogenes, and tumor suppressor genes contributes to these pathological alterations (Sheng *et al*., 2023).

Cyclooxygenase enzyme-2 (COX-2), a key inflammatory mediator, is expressed in various tissues and cells, potentially contributing to the initial stages of chronic liver illnesses and its metabolites playing a role in tumorigenesis. Increased COX-2-generated prostaglandin E2 (PGE2) levels promote cellular proliferation, migration, and angiogenesis, suppressing apoptosis and immune response. COX-2 inhibitors decrease COX-2 and down-regulate nuclear factor kappa-beta (NF-kB) linked to liver inflammation and hepatocellular carcinoma (HCC) development (Karim *et al*., 2022).

Moreover, caloric restriction (CR), frequently referred to as "undernutrition sans malnutrition," has shown a commitment to lowering the incidence of cancer besides its proven outcome of lengthening life. For instance, 30 % CR proved to defer carcinogenesis and reduce nitric oxide (NO)

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FUNDING Deanship of Research and Graduate Studies at King Khalid University, Abha, Saudi Arabia. Grant RGP2/312/45.

Received: 2024-06-17 Accepted: 2024-08-10

generation in p53-deficient mice. CR could also reduce the levels of inflammation biomarkers in postmenopausal women, and this result was meaningful since a greater risk of certain malignancies has been attributed to elevated levels of inflammatory biomarkers (Lv *et al*., 2014).

Another beneficial approach currently recognized to extend a rodent's life span and quality is reduced-function mutations in the insulin/insulin-like growth factor I (IGF-I) trajectory. Owing to the IGF-I anti-apoptotic effects, high levels of IGF-I constitute a legitimate mechanism of carcinogenesis. Furthermore, in human cancer cells, IGF-I was reported to boost the transcriptional activity of COX-2 and the stability of its mRNA (Stoeltzing *et al*., 2007).

According to Lettieri-Barbato *et al*. (2016) results, a discernible drop in circulating IGF-I levels in humans with different regimens of diet restriction was evident. This prompted us in the present work to investigate the effectiveness of 30 % CR either alone or combined with COX2 inhibitor (Celecoxib) on the pathogenesis of HCC in rats and to assess the possibility of suppressing the COX2 levels secondary to diet-mediated regulation of insulin growth factors (IGFs) (Lettieri-Barbato *et al*., 2016).

MATERIAL AND METHOD

Ethical approval. The experiment was conducted in strict accordance with the 06U Research Ethics Committee regulations at October 6 University, Cairo, Egypt after being approved (IRP: PRE-Me 2310001).

Experimental animals. In the present research, forty adult male albino rats (weight: 150-200 grams/age: 10-12 weeks) were enrolled. For a duration of seven days, the animals were allowed to adjust to standard living circumstances including temperature of 25 ± 2 ºC, humidity (40-70 %), and normal light and dark rhythm. Before the trial began, rats were housed in wire mesh cages with unrestricted food and water supply unless specified. The ad libitum rats consumed approximately 79 kcal daily. The ingredients of the standard food pellet were as follows: 29 % protein, 6 % fat, and 65 % carbohydrates generating about 3.3 kcal/g. After that, the rats were equally sorted into four distinct groups at random.

Group I (Control); in which animals were supplied with an intraperitoneal (i.p) isotonic saline (0.1 ml once), and then isotonic saline (3 ml/kg) was injected subcutaneously (Sc) once/week/6 weeks. Group II (HCC); rats received diethylnitrosamine (DEN) (200 mg/kg, single dose, i.p) accompanied by carbon-tetrachloride (CCl4) (3 ml/kg, Sc, once a week for 6 consecutive weeks) (Abdel aziz *et al*., 2011). Group III (CR); in which animals with induced HCC were supplied with a caloric-restricted diet (only provided with 70 % of that was given to controls). Group IV (CR-Celecoxib); represents the induced HCC animals supplied with a 30 % CR regimen (Ciobanu *et al*., 2017), in parallel with the administration of COX2 inhibitor (Celecoxib) at a fixed dose of 3.6 mg via gastric gavage twice/day (Bassiouny *et al*., 2010). All the experimental regimens in all groups were provided for 6 consecutive weeks. The rats subjected to CR were confined in individual cages for accurate monitoring of their food and water intake.

Samples collection and scarification. At the end of the experiment duration, blood was taken from the retro-bulbar plexus. Using the centrifuge, the plasma was obtained and then stored at ≤ -20 ˚C for further analysis of the insulin-like growth factor-I (IGF-I) and- II (IGF-II) as well as Alpha-fetoprotein (AFP) levels.

Right after the animals' cervical dislocation sacrifice, the livers were extracted and separated into two sections one was utilized for molecular analysis while the other was used for histological examination.

Chemicals. All the chemicals: COX2 inhibitor (celecoxib), Diethyl nitrosamine (DEN) solution (diluted 1:1 with castor oil) and Carbon-tetrachloride (CCl4) solution were bought from Sigma-Aldrich Egypt; Number C1900. The celecoxib dose was calculated using Paget`s table, a 70 kg-human dose is 200 mg twice a day. Considering this, a 150 g rat's daily dosage was 200×0.018= 3.6 mg (Bassiouny *et al*., 2010).

Measured parameters

Serum biochemical parameters

Measurement of serum Alpha-fetoprotein (AFP), Insulin-like growth factor-I (IGF-I), and Insulin-like growth factor-II (IGF-II). ELISA kits were utilized to measure both serum AFP (KAMIYA BIOMEDICAL) and IGF-II (Catalog No. EK0380) following the manufacturer's instructions. A rat anti-IGF-I monoclonal antibody was utilized to evaluate the serum IGF-I level as per the supplier's guidelines.

Measurement of liver molecular parameters

Quantitative real-time-polymerase chain reaction (qRT-PCR) assessment of liver tissue cyclo-oxygenase2 (COX2) and interleukin-6 (IL-6) gene expression. By applying an SV total RNA isolation system (Promega, Madison, WI, USA), the entire ribonucleic acid (RNA) was isolated from the liver specimens and refined. Using spectrophotometry, the resulting RNA was evaluated at 260 nm. Afterward, an RT-PCR kit (Stratagene, USA) was utilized to reverse the extracted RNA

Table SI. Primers of COX-2, IL-6 and β-actin.

	Forward	Reverse
COX-2	5' GC AAA TCCTTGCTGTTCCAATC 3'	5' GGAGAAGGCTTCCCAGCTTTTG 3'
IL-6	5' GAAACCGCTATGAAGTTCCTCTCTG-3'	5' TGTTGGGAGTGGTATCCTCTGTGA-3'
β-actin	5' TCT GGC ACC ACA CCT TCT ACA ATG 3'	5' AGC ACA GCC TGG ATA GCA ACG 3'

Abbreviations: COX2: Cyclo-oxigenase2, IL-6: Interleukin-6

into complementary deoxyribonucleic acid (cDNA). The primers for measured genes are mentioned in Table SI.

Histopathological examination

Histological stains. Sections of the paraffin-fixed liver specimens were prepared for histological evaluation using various stains including Hematoxylin and Eosin (H&E), Masson's trichrome (MT), and Periodic Acid Schiff (PAS).

Immunohistochemical evaluation of caspase 3. The liver tissue samples underwent avidin-biotin processing. The primary antibody was rabbit polyclonal anti-caspase 3 antibody (ab4051) (1:100, IHC-P, Abcam®, Cambridge, MA, USA) while the goat anti-rabbit IgG H&L (HRP) (ab205718) was the secondary antibody. In 6 not-overlapping areas for each slide from the respective group, the area % of caspase 3 immuno-expression in the liver sections was assessed via image j analysis software (Media Cybernetics, Silver Spring, Maryland, USA).

Statistics. The data was analyzed using SPSS version 16, with ANOVA and Bonferroni multiple comparisons *post hoc* tests for normally distributed variables and non-parametric Kruskal-Wallis and Mann-Whitney tests for non-normally dispersed variables. Pearson correlation coefficient was used to assess correlations between variables, with p-values <0.05 indicating statistical significance.

RESULTS

Attenuated serum levels of AFP, IGF-I, and IGF-II secondary to CR and Celecoxib. As shown in Table I, the study found a significant increase in serum levels of AFP, IGF-I, and IGF-II in the HCC group compared to the control group. Despite a decline in AFP, IGF-I, and IGF-II levels in either the CR or CR-Celecoxib groups, statistical significance was still present compared to the control group. The combined CR-Celecoxib regimen was found to be superior to CR alone in terms of IGF-II.

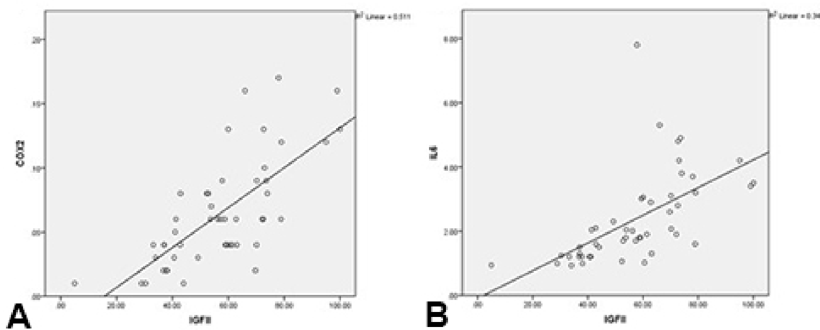


Fig. 1. Correlation between IGF-II and inflammatory biomarkers COX-2 and IL-6.

Improved liver tissue gene expression of COX2 and IL-6 secondary to CR and Celecoxib. As displayed in Table I, COX2 and IL-6 gene expression levels increased in HCC livers compared to control tissues. However, COX2 and IL-6 gene expressions declined in both CR and CR-Celecoxib groups, with IL-6 levels mostly normalizing in CR-Celecoxib and partial improvement in CR.

Correlation. A significantly (p <0.001) strong positive correlation between the IGF II and the inflammatory biomarkers COX2 and IL-6 (r: 0.701, 0.745, 0.715 and 0.590 respectively) was detected as shown in Figure 1.

Histological results

Table I. Serum biochemical and liver molecular parameters among groups.

	Control	HCC	CR	CR + Celecoxib
Serum biochemical parameters				
Alpha fetoprotein (ng/dl)	0.53± 0.09	1.98± 0.45*	0.91± 0.15*#	0.82± 0.19*#
Insulin like growth factor-I (pg/ml)	31.19± 3.43	73.89± 15.39*	51.18± 6.5*#	43.6± 10*#
Insulin like growth factor-II	33.59±9.9	77.34±14*	65±8.68*#	51.73±7.8*#$
Liver tissue gene expression				
Cyclooxygenase-2	0.025± 0.014	0.123± 0.29*	0.052± 0.019*#	0.055± 0.17*#
Interleukin-6	1.18±0.19	4.3±1.3*	2.28±0.59*#	1.66±0.37#

*: Significant relative to control, #: Significant relative to HCC, $: Significant relative to CR. All data were represented as mean± SD. p< 0.05 was significant.

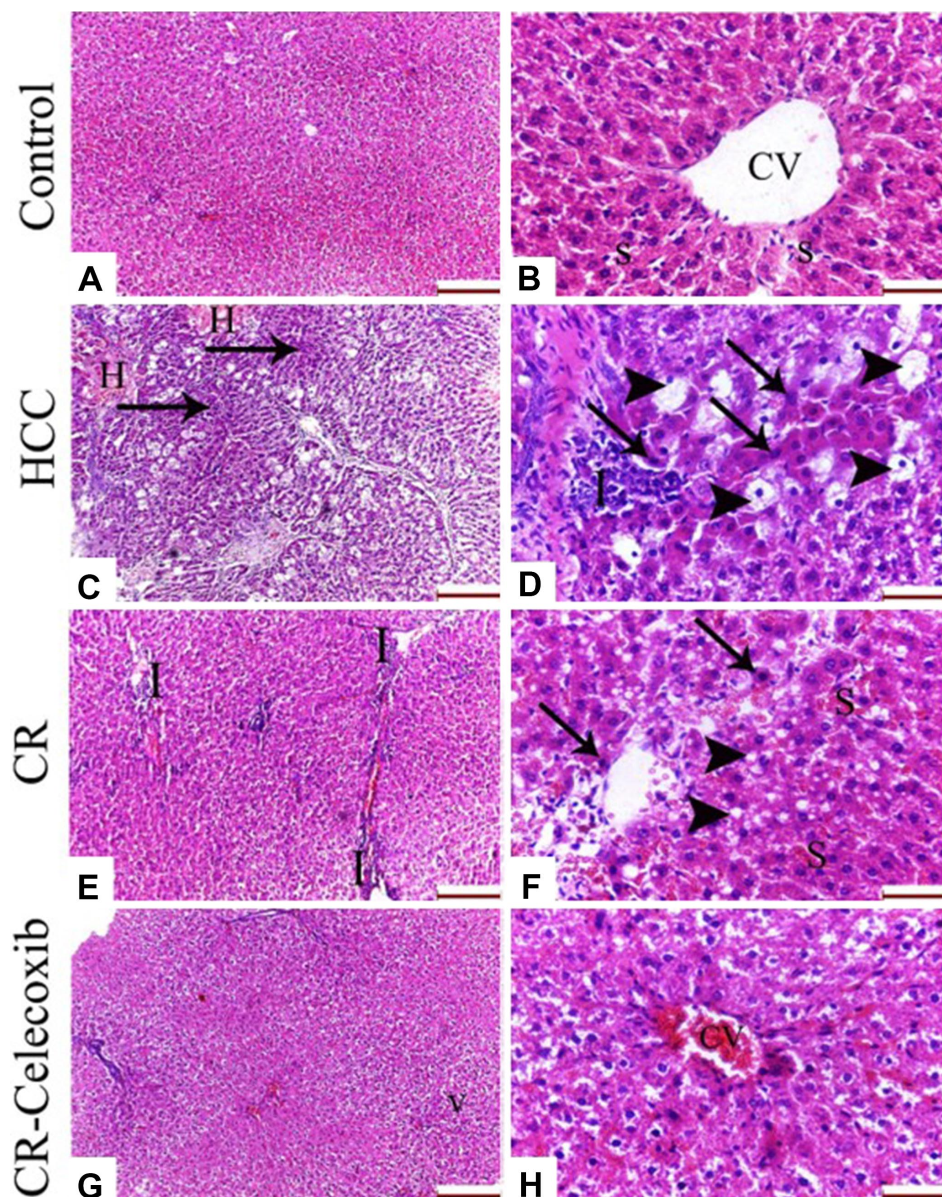


Fig. 2. H&E-stained liver sections of (A) Control group exhibiting regular hepatic pattern. (B) Further magnification of control group reveals densely packed cords of polygonal hepatocytes containing rounded vesicular nuclei and acidophilic cytoplasm emanating from central vein (CV) and partitioned by blood sinusoids (S) which consist of healthy endothelial cells with collapsed nuclei and irregularly distributed, dispersed Kupffer cells with ovoid nuclei. (C) HCC group showing large dysplastic hepatocytes (arrows) in a cirrhotic liver with patches of hemorrhagic necrosis (H). (D) Greater magnification of the HCC sections showing sheet arrangement of neoplastic hepatocytes with scattered mitotic figures and nuclear atypia. The neoplastic hepatocytes display pleomorphism, increased nuclear to cytoplasmic ratio (N/C), and hyperchromasia (arrows). Hepatocytes with ballooning degeneration (clear cell changes) (arrowheads) and inflammatory cellular infiltration (I) are also observed. (E) Group 3 (CR) showing infiltration of the fibrotic septae by mononuclear inflammatory cells (I) (F) CR group at higher magnification displaying hepatocytes with a little elevated N/C ratio (arrows), limited clear cell change (arrowheads) and sinusoidal congestion (S). (G) Group 4 (CR-celecoxib) revealed maintained hepatic architecture with minimal hepatocyte desmoplasia and mild vacuolation (V). (H) Higher magnification of the CR-celecoxib group depicts hepatocytes with less nuclear atypia and a slight central vein (CV) congestion (scale bar: 200µm for A, C, E, and g, and 50µm for B, D, F, and H).

H & E-stained liver sections results. H&E-stained sections of the HCC group livers showed sheets of neoplastic hepatocytes with features of large cell dysplasia in a cirrhotic liver with areas of hemorrhagic necrosis, scattered mitotic figures, and nuclear atypia. The neoplastic hepatocytes displayed pleomorphism, increased nuclear to cytoplasmic (N/C) ratio, and hyperchromatosis. Hepatocytes with ballooning degeneration (clear cell changes) and inflammatory cellular infiltration were also observed. The CR group showed a slight increase in N/C ratio, few regions of clear cell change, and sinusoidal congestion, as well as mild mononuclear inflammatory cell infiltrates in the fibrotic septa. The CR-celecoxib group revealed maintained hepatic architecture, and minimal hepatocyte desmoplasia, along with mild vacuolation and congestion of the central vein (Fig. 2).

MT-stained liver sections results. MT-stained sections of the HCC group liver tissues revealed thickened collagen bundles surrounding micronodules with central to portal bridging fibrosis and marked interstitial collagen deposition. On the other hand, the CR group showed moderate perivascular and interstitial collagen deposition. However, the CR-celecoxib group revealed obvious regression in hepatic fibrosis where only mild collagen deposition around the central vein and minimal fibrous expansion of portal tracts were observed. Also, thin collagen fibers were seen surrounding the hepatocytes and the clear cells in the hepatic parenchyma (Fig. 3).

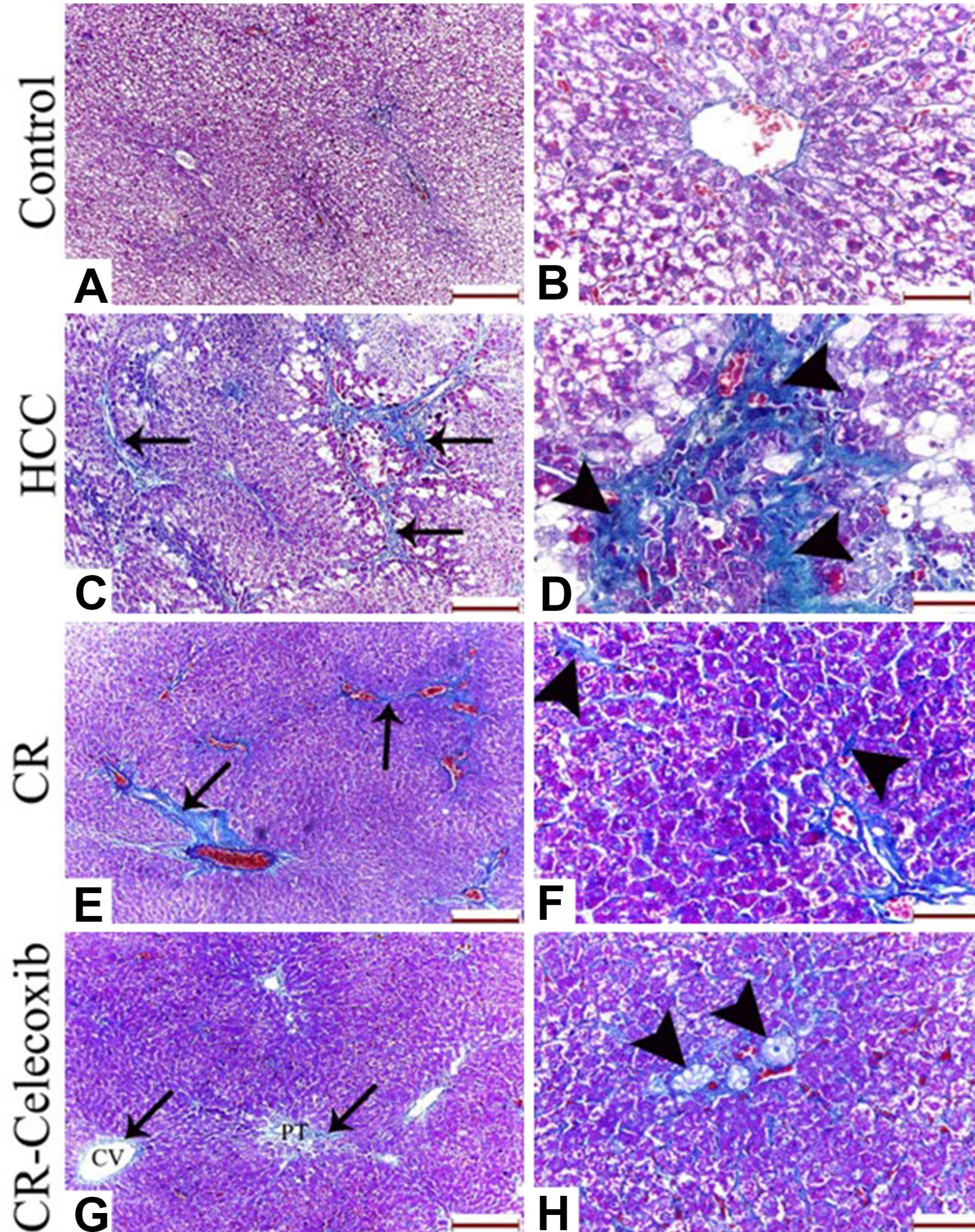


Fig. 3. Masson's trichome-stained liver sections of: (A, B) Control group. (C, D) HCC group demonstrating thickened collagen bundles (arrows) surrounding micronodules with central to portal bridging fibrosis and marked interstitial collagen deposition (arrowhead) (E, F) CR group showing moderate perivascular (arrows) and interstitial (arrowheads) collagen deposition (G, H) CR-celecoxib group exhibits a slight fibrous extension of the portal tracts (PT) and minor deposits of collagen (arrows) surrounding central vein (CV). Collagen fibers surround the hepatocytes and clear cells (arrowheads) in the parenchyma (Scale bars: 200µm for items A, C, E, and G; 50µm for items B, D, F, and H).

PAS-stained liver sections results. PAS staining of the HCC group liver sections revealed a significant decrease in the liver glycogen. This was evidenced by presence of glycogen-depleted hepatocytes (Clear cells) in the parenchyma and areas encircling the central vein. The PAS-positive hepatocytes were primarily retrieved from the vicinity of the portal tract, as observed in the CR group. The parenchyma of the CR-Celecoxib group livers displayed significantly widespread positive PAS-stained hepatocytes with robust glycogen recovery (Fig. 4).

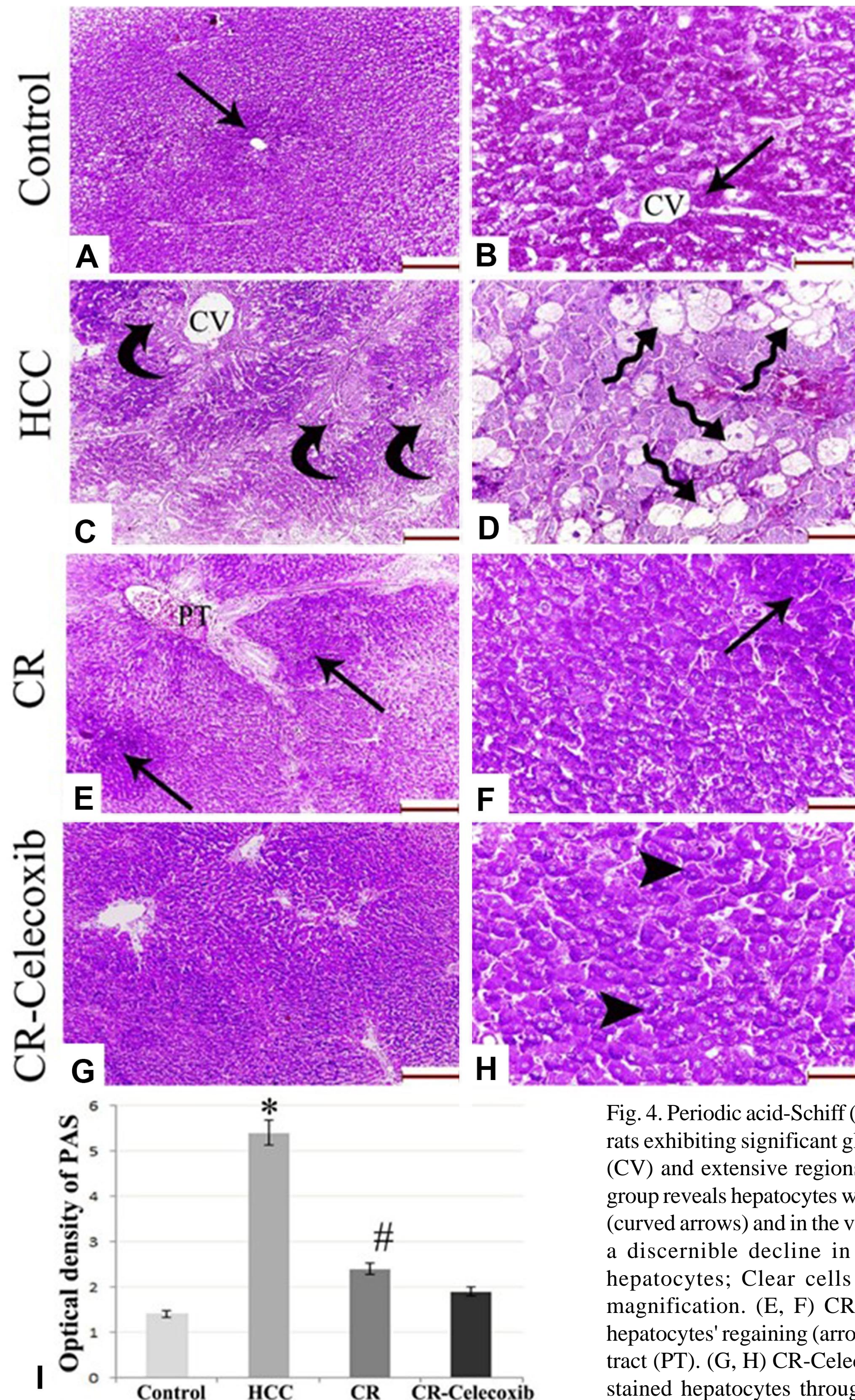
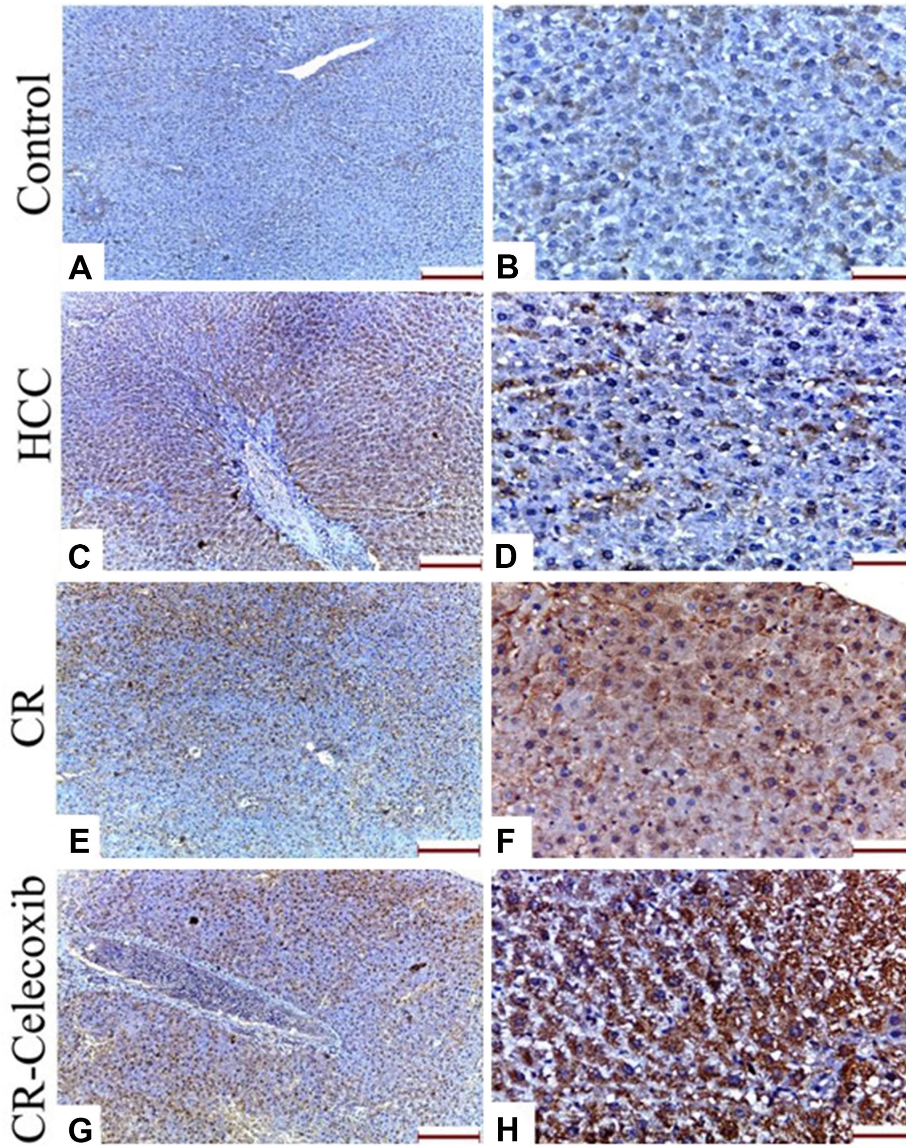


Fig. 4. Periodic acid-Schiff (PAS) stained liver tissues of: (A, B) Control rats exhibiting significant glycogen (arrows) enclosing the central vein (CV) and extensive regions of PAS-positive hepatocytes. (c,d) HCC group reveals hepatocytes with a mild PAS reaction in liver parenchyma (curved arrows) and in the vicinity of central vein (CV) which indicated a discernible decline in glycogen density. Glycogen-depleted hepatocytes; Clear cells (spiral arrows) are obvious at higher magnification. (E, F) CR group demonstrating the PAS-positive hepatocytes' regaining (arrows), primarily in near-by areas of the portal tract (PT). (G, H) CR-Celecoxib group showing strong positive PAS-stained hepatocytes throughout the liver parenchyma. At a greater magnification (H), glycogen (arrow heads) is obviously restored (scale bar: 200µm for A, C, E, and G; scale bar: 50µm for B, D, F, and H).

Immunohistochemical (IHC) results

Liver Caspase 3. The liver tissues of the HCC group showed moderate IHC expression of caspase 3 compared to control. However, an intense caspase 3 IHC with a corresponding statistically significant increase in the mean area percent of caspase 3 in the CR and CR-celecoxib groups relative to the HCC group was noted (Fig. 5).



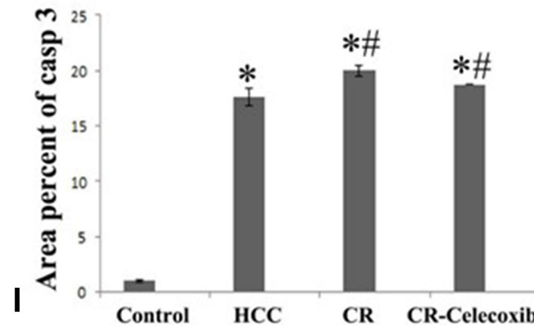


Fig. 5. Caspase 3 immunohistochemically-stained liver sections of: (A, B) Control group. (C, D) HCC group. (E, F) CR group. (G, H) CR-celecoxib group. (i) Area percent of caspase 3 immunohistochemical expression among groups (n=6) *: Statistically significant compared to control, #: Statistically significant compared to HCC ($p<0.05$ was considered significant) (scale bar: 200µm for A, C, E, and G; scale bar: 50µm for B, D, F, and H).

DISCUSSION

Liver carcinogenesis is a multifaceted process involving inflammation, tissue destruction, regeneration, uncontrolled proliferation, and genetic shifts that lead to malignant behavior in hepatocytes. Hepato-carcinogenesis can be created through weekly injections of CCL4 after a single injection of DEN, a robust carcinogen. DEN's bio-activation by CYP2E1 results in severe oxidative burden and DNA adducts, leading to genetically distorted hepatocytes. Reactive metabolites of CCl4 in the liver can cause lipid/protein peroxidation, covalent attachment to proteins, and increased intracellular cations which negatively impact cells contributing to tumorigenesis (Abdul-Azeez & Mutlag, 2023).

IGF-I concentrations in tumor microenvironments promote carcinogenesis and rapid tumor growth through anti-apoptotic signaling and metabolic shift via the PI3K–AKT–mTORC1 pathway (Galal *et al*., 2023). Also, IGF-II overexpression has been implicated in hepato-carcinogenesis. Wu & Zhu (2011) depicted that IGF-I and IGF-II bind to IGF-I receptor (IGF-IR) hence promoting tumor cell motility and triggering anti-apoptosis, contingent on the tumor cell's reaction to p53.

The IGF-II/insulin receptor-A (IR-A) loop, through phosphorylation and inhibition of p53, promotes cancer cell growth, migration, and self-renewal, thereby activating transcription of Oct-4 and Nanog factors, which are linked to cancer progression and poor prognosis (Galal *et al*., 2023). According to Benabou *et al*. (2019) AFP is linked to enhanced IR-A in HCC. Epithelial discoidin domain-containing receptor 1 (DDR1) is a crucial regulator of IGF-1R and IR function, and dysregulation of DDR function has been linked to cancer by altering the IGF-II/IR-A loop. IGF-II overexpression in HCC is partly due to demethylation of fetal-specific promoters, which activates IGF1R signaling, accelerating liver tumor growth with hepatic expression of MYC and AKT1 oncogenes (Martinez-Quetglas *et al*., 2016).

Overexpression of IGF-1R can lead to the loss of tumor suppressors like p53, NRF2, and reduced insulin-like growth factor binding protein-3 (IGFBP-3) due to proteolysis in late cancer stages. Activated IGF-1R also benefits the epithelial-mesenchymal transition (EMT) and integrin stabilization, potentially contributing to cancer dissemination. IGFRs enhance chemoresistance by avoiding the full-blown effects of anti-cancer medications targeting DNA (Galal *et al*., 2023).

Inflammation is a precursor to HCC and liver fibrosis, with a significant increase in transcriptional levels of COX-2 and IL-6 in liver. COX-2 produces prostaglandins (PGs) and thromboxane which are crucial for inflammation-tumor transformation. COX-2 enhances vascular endothelial growth factors (VEGF) dependent tumor angiogenesis, drives anti-apoptosis through Bcl-2 and protein kinase B signaling, and significantly affects tumor invasiveness through matrix metalloproteinases. COX-2 overexpression via IGF-II/IGF-I receptor pathway in Caco-2 colon cancer cells was a biochemical predictor of HCC in advanced malignancies with negative prognosis or recurrences.

IL-6, a pro-inflammatory cytokine, is crucial for the onset and progression of HCC due to its anti-apoptotic and pro-angiogenic properties. Elevated IL-6 is more pronounced in HCC patients significantly higher than cirrhosis patients. Naugler & Karin (2008) confirmed that persistent inflammation with elevated IL-6 levels is responsible for DEN-induced HCC.

IL-6 stimulates JAK-STAT3 and Ras/Raf/MAPK signaling, enhancing HCC cell viability and proliferation by inducing apoptosis-related genes like Bcl-2, Bcl-xl, Survivin, Mcl-1, and XIAP. It IL-6 enhances tumor angiogenesis and induces EMT, enabling cancer cell dissemination. Also, IL-6 inhibits Treg differentiation and promotes Th17 differentiation, stimulates the PI3K/PKB/Akt pathway, thereby activating NF-κB (Wang *et al*., 2011).

CR reduces serum IGF-I by about 40 %. CR can reduce liver metastasis by suppressing IGF-I and IGF-1R through miR-29 and miR-30 overexpression and downregulation of IGFI/AKT/mTOR signaling pathway. Dampening IGF-I by CR results in activating transcription factors FoxO1, FoxO3, and Nrf2, thus reducing inflammation and cancer, and suppressing the activity of rapamycin molecular target. Also, IGF-II inhibition downregulates VEGFA and halts the proliferation of human colorectal cancer cells (Fontana & Klein, 2007).

CR boosts anti-inflammatory mechanisms, potentially suppressing the damage-associated molecular pattern molecule inflammasome, as the sensitivity of GH-IGF-I signals evolves Also, CR increases the anti-inflammatory driver Suppression of Cytokine Signaling 3 which inhibits the synthesis of IL-6, a crucial component for COX-2 production (De Luca *et al*., 2024).

NF-κB is a fundamental transcription factor for pro-angiogenic and anti-apoptotic attributes of malignant brain tumors. Extremely aggressive gliomas have greater COX-2 expression triggered by NF-κB. CR induces reduction in CD68 macrophages which are the dominant source of tumor necrosis factor (TNF-α), thus suppressing NF-κB activation

in CT-2A tumor tissue microenvironment (Csiszar *et al*., 2009).

The impact of combined CR-celecoxib regimen was more effective against hepatocarcinogenesis. Song *et al*.'s study showed HCC regression after a 3-month COX-2 inhibitor therapy for degenerative arthritis. IGF-I, a potent mitogen, influencing early carcinogenesis by promoting angiogenesis via COX-2/PGE/EP3/VEGF signaling. This leads to mast cell release enhancing pro-tumorigenic remodeling in the stroma (Maltby *et al*., 2009). However, Celecoxib was reported to markedly suppress the mast cell's IGF-I/COX-2/PGE2/EP3 chemotactic pathway within the tumor. Research revealed that celecoxib significantly reduces the production of IGF-II, a key growth factor linked to HCC, indicating its potential anti-oncogenic properties. Also, Celecoxib inhibits non-small cell lung cancer cell proliferation and invasion by suppressing IGF-1R phosphorylation, upregulating IGFBP-3 expression, and downregulating the PI3K/AKT (Hamzawy *et al*., 2015).

COX-2 inhibitors cause proapoptotic activity in tumor cells. Anti-apoptotic genes Bcl-2, Bcl-XL, and Survivin are targets of IL-6/STAT3, potentially increasing apoptosis in cancer. Celecoxib may prevent STAT3 stimulation in HCC by preventing JAK2 phosphorylation. Furthermore, Celecoxib can cause apoptosis in prostate cancer cells by inhibiting the anti-apoptotic AKT activation which primarily phosphorylate and inactivate BAD and caspase 9 as well, its effect on caspase 3 was reported in our study. Though Celecoxib suppressed PDK1 and PTEN phosphorylation in cholangiocarcinoma cells, the exact mechanism by which it reduces AKT phosphorylation is not yet fully understood (Wu *et al*., 2004).

Human HCC cells overexpress Bcl-xL and Survivin, inhibiting apoptosis and promoting resistance to tumor necrosis factor-related apoptosis, inducing ligand (TRAIL)-induced apoptosis. COX-2 inhibitors downregulate these factors, sensitizing HCC cells to TRAIL. Von Hippel Lindau (VHL) protein plays a role in ubiquitinating hypoxia-inducible factor-1α (HIF-1α) and downregulating VEGF. VHL protein is more detectable when COX inhibitors are administered. COX inhibitors, being agonists for peroxisome proliferator-activated receptors (PPARs) α and γ, exhibit potent anti-inflammatory and anti-angiogenic properties beyond cancer's cell-autonomous activities (Yamanaka *et al*., 2006).

Pyruvate kinase M2 isoform (PKM2) plays a crucial role in aerobic glycolysis and controls apoptosis. COX-2 knockdown significantly reduces PKM2 and HIF-1α expression in HCC tissues, suggesting metabolic disruptions may contribute to COX-2-induced apoptosis resistance (Wang *et al*., 2019).

Tumorigenesis leads to increased aerobic glycolysis and depletion of glycogen in hepatocellular metabolic machinery. This results in the formation of clear cells. Celecoxib's potency in restoring hepatic glycogen is attributed to its anti-fibrotic effect. The amelioration of fibrosis leads to improved blood flow and metabolism (Chávez *et al*., 2010), as demonstrated by PAS staining.

CONCLUSION. Adjuvant non-cytotoxic interventions targeting IGFs, COX-2, and IL-6, like CR or Celecoxib with conventional HCC therapies could increase survival rates.

ACKNOWLEDGEMENTS. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through a Large Research Project under grant number RGP2/312/45.

SHAMSELDEEN, A. M.; EL HAMZAWY, M.; ABOULHODA, B. E.; RASHED, L; ALGHAMDI, M. & HASSAN, F. E. Efecto sinérgico de la restricción calórica y el inhibidor de la ciclooxigenasa-2 "Celecoxib" contra los IGF/COX-2 que median el carcinoma hepatocelular inducido químicamente en ratas. *Int. J. Morphol., 42(6)*:1628-1637, 2024.

RESUMEN: La restricción calórica (RC), junto con su capacidad para aumentar la longevidad, ejerce efectos inhibidores contra varios tumores. El propósito de esta investigación actual fue investigar las posibles implicaciones de la RC, ya sea de forma independiente o junto con un inhibidor de la ciclooxigenasa 2 (Celecoxib), en la fisiopatología del carcinoma hepatocelular inducido (CHC). Cuarenta ratas Wistar macho adultas se dividieron en cuatro grupos: Control, CHC; inducido mediante inyección intraperitoneal de dietilnitrosamina, luego se le administró una inyección subcutánea semanal de tetracloruro de carbono (CCl4) una vez por semana durante 6 semanas consecutivas, grupo CR y grupo CR-Celecoxib. Los resultados revelaron niveles mejorados de factor de crecimiento insulínico sérico I (IGF-I) y II (IGF-II), junto con un aumento significativo en la expresión de los genes COX-2 e IL-6, así como aceleración de cambios patológicos, depleción de glucógeno, fibrosis mejorada y disminución de la expresión de caspasa 3 en el hígado. Sin embargo, tanto IGF-I, II como alfa-fetoproteína (AFP) disminuyeron significativamente en el grupo CR co-tratado con Celecoxib, lo que se reflejó positivamente en la arquitectura hepática. Concluimos que la inhibición combinada de CR y COX-2 interfiere con la patogénesis del CHC.

PALABRAS CLAVE: Carcinoma hepatocelular; COX2; IGF; Celecoxib; Restricción calórica.

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