Developmental Expression of Cyclooxygenase isoforms in the hepatocytes of Albino Rat

Original Article

Sara Mohammed Naguib Abdel-Hafez, Saadia Ragab Said, Soha Abdelkawi Abdel wahab

Departments of Histology, Faculty of Medicine, Minia University, Egypt.

ABSTRACT

Background: The liver represents the major organ that participates in the elimination and degradation of arachidonic acid of systemic origin. This study is one of few types of research that studied the detailed expression of cyclooxygenase in the hepatocytes and other cells in liver tissue at different stages of maturation.

Aim: The present study is concerned with exploring and compare the developmental expression of cyclooxygenase (COX-1, COX-2, and COX-3) in the liver tissue using immunohistochemical techniques. This will open the future for a new rationale of the optimal therapeutic use of COX in liver diseases.

Materials and Methods: A total number of 8 embryos and 40 rats at different ages postnatal were used. The prenatal rats were 18 days old of gestation while the postnatal rats were at 1, 7, 14, 21, and 28 days old.

Results: The results showed that, in liver cells, the expression of COX isoforms was scientifically changed with the development of the liver tissue. COX-1 and COX-2 immunostaining were noticed to be the same in some ages however, the COX-1 expression resembled the COX-3 expression in other ages.

Conclusion: It can conclude the expression of three types of COX isoforms in liver tissue played an important role in the development and maturation of different cells population either through prostaglandin production or a novel gene production. Nuclear localization of COX in the hepatocytes arises a lot of questions about the role of COX in the enhancement of liver division and maturation.

Key Words: Cyclooxygenase, isoforms, liver, rat.

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Corresponding Author: Sara Mohammed Naguib Abdel-Hafez, MD, Department of Histology, Faculty of Medicine, Minia University, Egypt, **Tel.:** 00201061479147, **E-mail:** sara_histology@yahoo.com

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INTRODUCTION

The liver is composed of parenchymal cells, hepatocytes, and non-parenchymal cells, mainly Kupffer cells, hepatic stellate cells (HSC), and sinusoidal endothelial cells (SEC). It has been reported that, in the normal liver, SEC and Kupffer cells are the primary sources of COX-dependent eicosanoid production[1].

Cyclooxygenases (COX) catalyze the key regulatory step in the biosynthesis of prostaglandins and thromboxanes from membrane-derived arachidonic acid. There are two well-characterized COX isoforms, the "housekeeping" or "constitutive" isoform COX-1 and the "inducible" isoform COX-2. They differ markedly in their pattern of regulation and physiological function[2]. COX-3 is a third distinct COX isozyme, COX-3, and one of the PCOX-1 proteins (PCOX-1a) are made from the COX-1 gene but retain intron 1 in their mRNAs[3].

The prostaglandins formed by the enzymatic activity of COX-1 are primarily involved in the regulation of homeostatic functions throughout the body, whereas the prostaglandins formed by COX-2 primarily mediate pain[4] and inflammation[5].

The liver represents the major organ that participates in the elimination and degradation of arachidonic acid of systemic origin[6]. The present work is one of few types of research that studied the detailed expression of cyclooxygenase in the rat liver tissue at different stages of maturation.

This study aims to explore and compare the developmental expression of cyclooxygenase (COX-1, COX-2, and COX-3) in the liver tissue using immunohistochemical techniques. This will open the future for a new rationale of the optimal therapeutic use of COX in liver diseases.

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MATERIALS AND METHODS

Animals:

This study was based on biopsies taken from the liver tissues from normal male Sprague Dawley rats. A total number of 8 embryos (weighting 30 gm) and 40 rats at different ages postnatal were used. The rats which were used prenatal were 18 days of gestation while the ages of rats that used postnatal were 1, 7, 14, 21, 28 days old and weighed 50, 80, 120, 150, 170 grams respectively.

Male Sprague Dawley rats were singly housed in plastic cages and maintained in a light, humidity, and temperature-controlled environment for one week before the experiment. Standard rat diet and water were allowed. All animals were sacrificed at the same time of day. Then these animals were euthanized by decapitation under light halothane anesthesia. The uterus of pregnant animals was opened and the liver of the fetuses on the 18th day of prenatal life was removed (this age was detected by vaginal plug). The liver's offspring were rapidly removed and rapidly put in formalin (24 hours).

Histological and immunohistochemical study

Specimens were taken from the livers and fixed in 10% formol saline for 48 hours. After proper fixation, the specimens were dehydrated, cleared, and embedded in hard paraffin. Sections were cut (6µm) on a standard microtome. Then the sections were stained with hematoxylin and eosin for general histological study[7]. Additional slides were proceeded for the immunohistochemical study. Immunohistochemical staining was performed using monoclonal rabbit antibodies (COX_1, COX_2, and COX_3). Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue. Sections were deparaffinized with xylene, dehydrated in a graded series of alcohol solutions, and then washed in phosphate - buffer saline (PBS). Endogenous peroxidases were quenched by using 3% H2O2 for 15 min in methanol (Peroxidase blocking solution) followed by washing in Tris - buffer saline (TBS). Non-specific binding of IgG was blocked by adding normal goat serum, diluted 1: 50 in 0.1 % bovine serum albumin with TBS for 30 min. Diluted primary antibodies for anti-COX-1(Catalog number: ab227513; dilution: 1/100), anti-COX -2 (Catalog number: ab169782; dilution: 1/50) and anti-Cox-3 Catalog number: ab37269; dilution: 1-4 µg/ml) ; Abcam company were applied on the slides overnight at room temperature. Sections then were washed perfectly 3 times each for 5 minutes in buffer and incubated for further 30 minutes. with biotinylated goat anti-rabbit secondary antibodies diluted 1:1000, followed by washing. Following further 30 min. incubation with Vectastain ABC kits (Avidin, Biotinylated horseradish peroxidase Complex) and washing for 10 minutes, the substrate, diaminobenzidine tetra hydrochloride (DAB) in distilled water was applied for 5–10 minutes. then dehydrated by passing through ascending concentrations of ethanol then cleared with xylene. Coverslip using mounting media is put. This substrate gives a clear brown stain at the immune-reactive sites[8]. The positive control for anti-COX_1 antibody was mouse brain. The positive control for anti-COX_2 antibody was rat lymph node. The positive control for anti-Cox-3 antibody was lung carcinoma (not included). For negative control slides, the same steps, but without the primary antibody (not included).

Photography:

An Olympus (U.TV0.5XC-3) light microscopy was used. Slides were photographed using an Olympus digital camera. The mean area fraction of three types of COX expression was quantified in ten non-overlapping representative scattered fields from three sections of each rat of all groups as mentioned in[9] using image J 22 program (Image J 1.48 V, Wayne Rasband National Institutes of Health, USA)[10]

Statistical Analysis:

Results were expressed as means standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's post- analysis test was used to analyze the results for statistically significant difference. *P values <0.05* were considered significant. Two-way ANOVA was used to show an interaction between groups (*P values <0.0001* considered significant).

RESULTS

A) Hematoxylin and eosin stain results:

On the 18th day of prenatal life, the early liver primordial cells were characterized by a network of expanded sinusoids (irregular dilated blood spaces) that were separated from each other by primitive hepatic cords. These cords were formed of reticular meshwork of immature hepatocytes that interstices contained many hemopoietic cells (the progenitor cells of blood elements). The central veins were noticed to be lined by endothelial cells and some blood elements were observed inside their lumens (Fig. 1A). On the 1st postnatal day, a few hemopoietic cells were found in small clusters. Some eosinophils and some scattered lymphocytes were observed at this age. The Kupffer cells started to be recognized (Fig. 1B). On the 7th postnatal day, only few haemopoietic cells were found in small clusters. The hepatocytes showed an apparent increase in their size. AT this age the monocytes were observed along the liver sections (Fig. 1C). On the 14th postnatal day, it was noticed that the hemopoietic foci had completely disappeared from the liver tissue. An apparent increase in the hepatocytes size was also detected (Fig. 2A). On the 21st postnatal day, some hepatocytes had shown open face nuclei while others

showed dark nuclei (Fig. 2B). On the 28th postnatal day, the hepatocytes radiate like spokes of a wheel from the central veins, forming anastomosing fenestrated plates of liver cells, separated from each other by large vascular spaces named hepatic sinusoids (Fig. 2C).

B) Immunocytochemical results

1. Expression of cyclooxygenase 1 (COX-1) in normal liver tissue:

At the age of on the 18th day of prenatal life, positive signals for COX-1 showed the pattern of patchy distribution. It was obvious that high COX-1staining was markedly observed in the hematopoietic cells. The developing hepatocytes showed this expression especially in the cytoplasm and the perinuclear zone. The endothelial cells lining the central veins and the scattered lymphocytes were also showing this immunosignals (Fig. 3). On the 1st postnatal day, the level of COX-1 expression was apparent increased in the hematopoietic cells and endothelial cells lining the central veins more than observed in the previous group. In the hepatocytes, there was a translocation of perinuclear zone expression to be mainly nuclear. The dividing hepatocytes and the scattered lymphocytes remained to show this higher expression. At this age, the Kupffer cells started to show this COX-1 staining (Fig. 3B). On the 7th postnatal day, it is obvious that the expression of COX-1 was markedly increased in all cell's populations. The cytoplasmic expression in the hepatocytes was apparently increased. Some hepatocytes showed nuclear signals while others showed perinuclear zone expression. The endothelial cells, the Kupffer cells, and the scattered lymphocytes all were highly positive for COX-1 immunosignals (Fig. 4A). On the 14th postnatal day, a marked reduction of COX-1 immunosignals could be observed in all liver cells if compared to the high expression in the previous age. The hepatocytes, endothelial cells, and Kupffer cells all showed low COX-1 signals. The expression in the hepatocytes showed the pattern of heterogeneity; some hepatocytes had shown nuclear expression while others failed to take this type of expression (Fig. 4B). On the 21st postnatal day, again the expression of COX-1 was enhanced reaching its highest level. Hepatocytes showed high COX-1 cytoplasmic expression. Some hepatocytes showed nuclear signals while others showed perinuclear expression. Dividing hepatocytes were observed to take that type of expression. The endothelial, the Kupffer, and the lymphocytes cells all were highly positively stained (Fig. 4C). On the 28th postnatal day, the expression was mainly noticed in the Kupffer and endothelial cells. The hepatocytes showed perinuclear and weak cytoplasmic expression. Heterogeneity in the hepatocyte's expression was noticed; some hepatocytes had shown nuclear expression while others showed negative nuclear staining. The lymphocytes were observed to show high COX-1 staining (Fig. 4D).

2. Expression of cyclooxygenase 2 (COX-2) in normal liver tissue:

On the 18th day of prenatal life, positive signals for COX-2 expression were noticed in the hemopoietic cells while some expression was observed in the hepatocytes (Fig. 5A1). It could be seen this expression in the endothelial cells lining the central veins (Fig. 5A2). On the 1st postnatal day, the expression in the liver tissue showed a pattern of patchy distribution. The expression was hardly seen in the hemopoietic cells if compared to the high expression showed in the previous age. The hepatocytes showed cytoplasmic and perinuclear zone expression. The scattered lymphocytes and the Kupffer cells showed little COX-2 immunosignals (Fig. 5B). On the 7th postnatal day, the COX-2 staining was markedly enhanced in all cell populations (hemopoietic cells) more than in the previous age. The hepatocytes showed higher cytoplasmic and perinuclear staining. The endothelial cells lining the central veins, the Kupffer cells, and the lymphocytes all showed an apparent increase in the COX-2 staining (Fig. 5D). On the 14th postnatal day, the expression was remaining high in all cell's populations resembles the previous age. Hepatocytes were remained showing high cytoplasmic and perinuclear expression. The endothelial cells, the Kupffer cells, and the lymphocytes all showed high COX-2 signals (Fig. 6A). On the 21st postnatal day, there was a marked decline in the COX-2 signals in all cells population if compared to the high expression in the previous age. In the hepatocytes, the heterogeneity in the expression was noticed; some hepatocytes showed weak nuclear staining while others showed negative nuclear signals. There was some expression in the endothelial and the Kupffer cells (Fig. 6B). On the 28th postnatal day, the expression was mainly restricted to the endothelial cells (of the central veins and blood sinusoids) and the Kupffer cells (Fig. 6C).

3-Expression of cyclooxygenase 3 (COX-3) in normal liver tissue:

On the 18th day of prenatal life, positive signals for COX-3 were localized to the endothelial cells of the central veins. The hepatocytes showed negative immunosignals for COX-3 (Fig. 7a). On the 1st postnatal day, there was a high expression in the immature hemopoietic cells. The hepatocytes showed high cytoplasmic and perinuclear expression. The lymphocytes and the Kupffer cells all were positive for COX-3 expression. The COX-3 staining could be seen in the lymphocytes (Fig. 3B). On the 7th postnatal day, the expression in some hepatocytes was nuclear while others showed negative expression. The hemopoietic cells, endothelial, the Kupffer cells, and the dividing lymphocytes all showed higher COX-3 expression if compared to the previous group (Fig. 7B). On the 14th postnatal day, there was a translocation of the expression in the hepatocytes from the cytoplasm to be mainly nuclear. The dividing hepatocytes showed also

nuclear expression. The expression in the hepatocytes at this age was in the form of heterogeneity, that resembled the previous age expression. Endothelial cells lining the central veins and the Kupffer cells all showed higher COX-3 staining (Fig. 8B). On the 21st postnatal day, the hepatocytes were showing COX-3 expression in the form of heterogeneity, some hepatocytes nuclear expression while others showed a weak nuclear expression. On the other hand, some failed to show any nuclear expression. It was obvious that the COX-3 nuclear expression in hepatocytes was mainly observed in the centrilobular zone. The dividing hepatocytes, the endothelial, and the Kupffer cells all showed high COX-3 expression (Fig. 8C). On the 28th postnatal day, the expression in the hepatocytes was showing the pattern of heterogeneity which resembled the previous age. The expression in the hepatocytes became mainly perinuclear with weak cytoplasmic expression. The dividing hepatocytes, the endothelial, and the Kupffer cells all showed high COX-3 staining (Fig. 8C).

Results of morphometrical results

Three types of COX expression in the liver of all studied groups was observed. The expression of three types of COX was different from one type to another one; COX-1, COX-2, and COX-3 immunosignals were significantly changed with the development of the liver tissue. The COX-1 staining resembled COX-2 staining in some ages however it resembled COX-3 in other ages (Histogram 1).

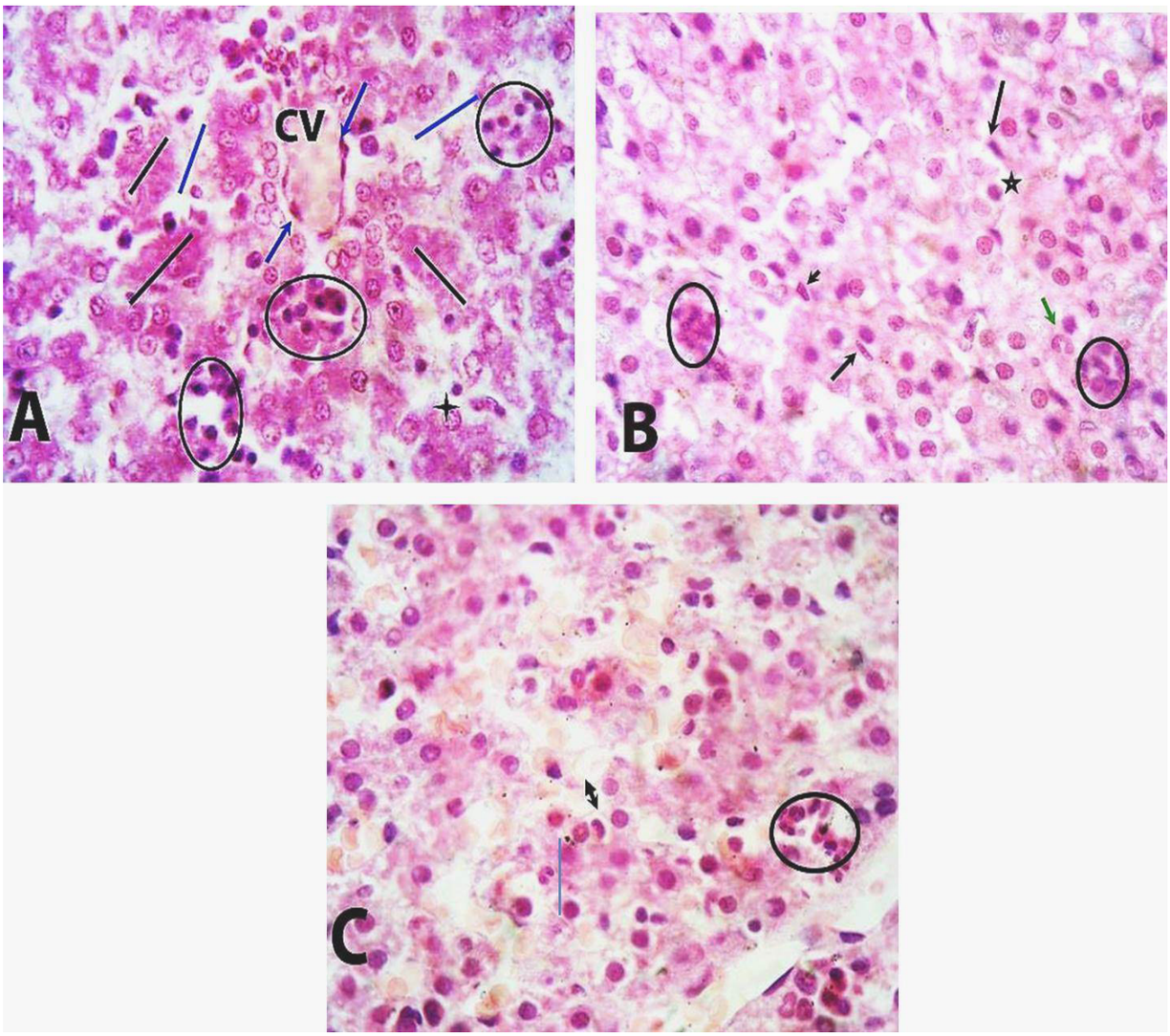


Fig. 1: Photomicrographs of rat's liver tissues at different age groups.
A) 18th day of prenatal life, showing the immature hepatocytes arrange in cords (black lines), separated by expanded blood sinusoids (blue lines). Notice the foci of hemopoietic cells foci (circles) and the scattered lymphocytes (star). Notice that the central veins (CV) are lined by endothelial cells (blue arrow).
B)1st postnatal day, showing apparent decrease in the hemopoietic cells (circles). Notice the appearance of eosinophils (green arrow) and Kupffer cells (black arrows).
C) 7th postnatal day, showing few hemopoietic cells in a small cluster (circle) and monocytes (double arrow) (HX&E X 400).

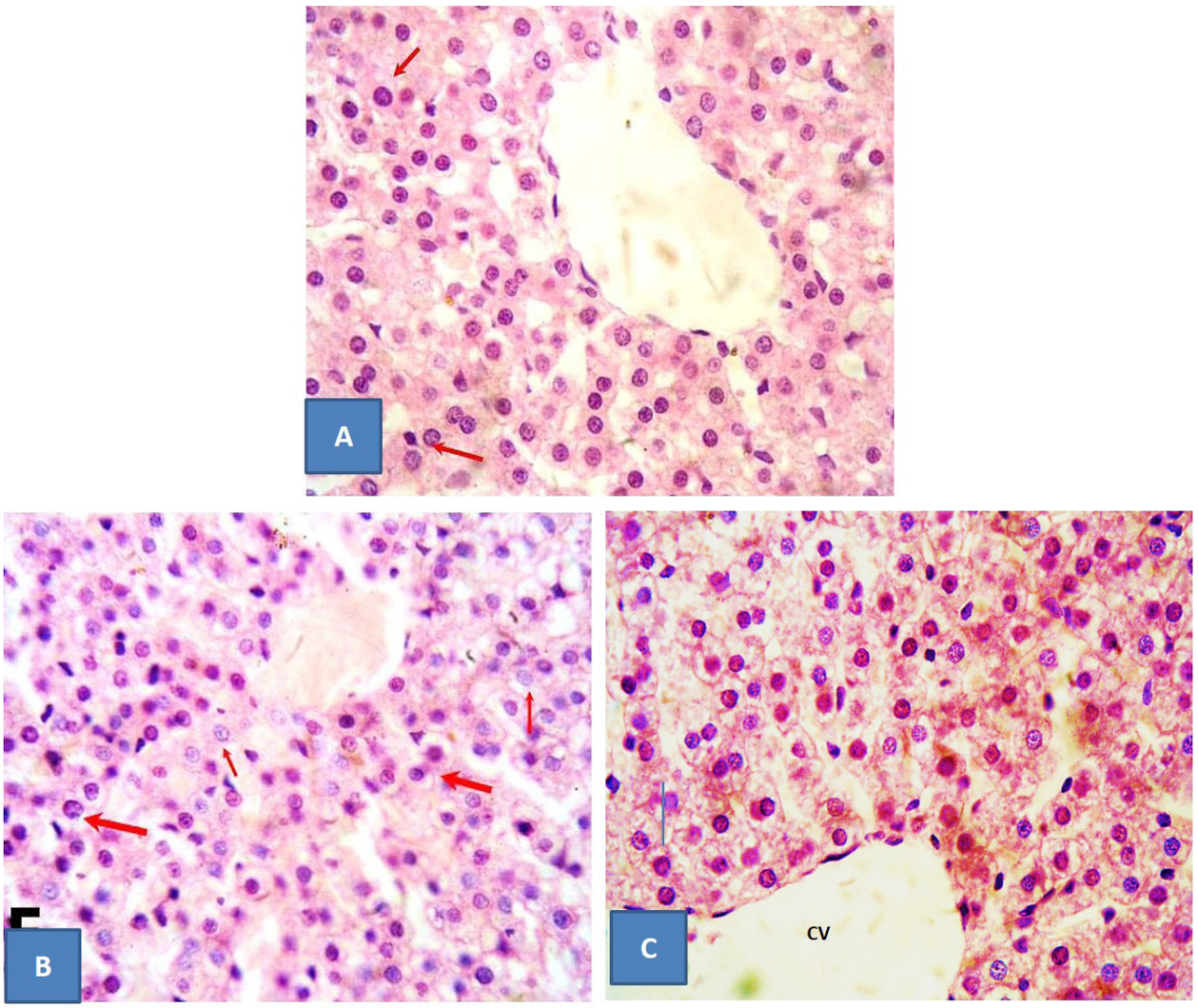


Fig. 2: Photomicrographs of rats' liver tissues at different age groups:
A) 14th postnatal day, showing complete absence of the hemopoietic cells. Notice the apparent increase in hepatocytes size (red arrows).
B) 21st postnatal day, showing that some hepatocytes have open face nuclei (thick red arrow) while others have dark nuclei (thin red arrow).
C) 28th postnatal day showing that the hepatocytes radiate like spokes of a wheel from the central vein (CV), separated by blood sinusoids (blue line). (HX&E X 400).

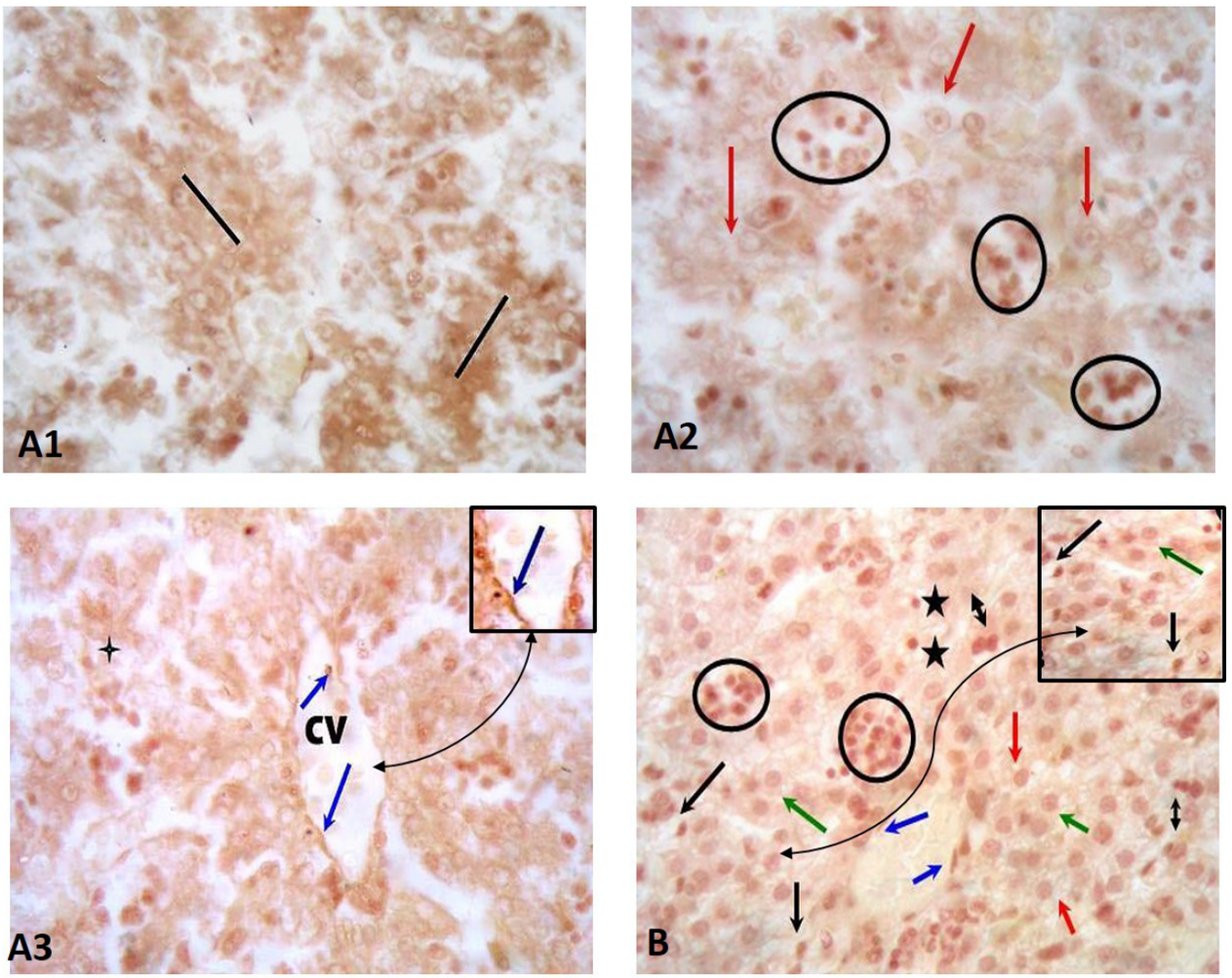


Fig. 3: Photomicrograph of the immunohistochemical localization of COX-1 in a rat liver tissue: A) The 18th day of prenatal life, A1) showing the expression in the hepatocytes takes the pattern of patchy distribution (lines).
A2) showing the high expression in the hemopoietic cells (circles). Notice the perinuclear expression in the hepatocytes (red arrows).
A3) Showing the COX-1 staining in the endothelial cells (blue arrows) lining the central veins (CV) and in the scattered lymphocytes (star). Magnification X400.
B) The 1st postnatal day, showing the increase in the COX-1 expression in the immature hematopoietic cells (circle). Notice the nuclear and cytoplasmic expression in the hepatocytes (red arrows). The endothelial cells, hepatocytes (green arrows), scattered lymphocytes (stars) all show the expression. Notice also the COX-1 expression in the Kupffer cells (black arrows). (X 400; insets X 1000).

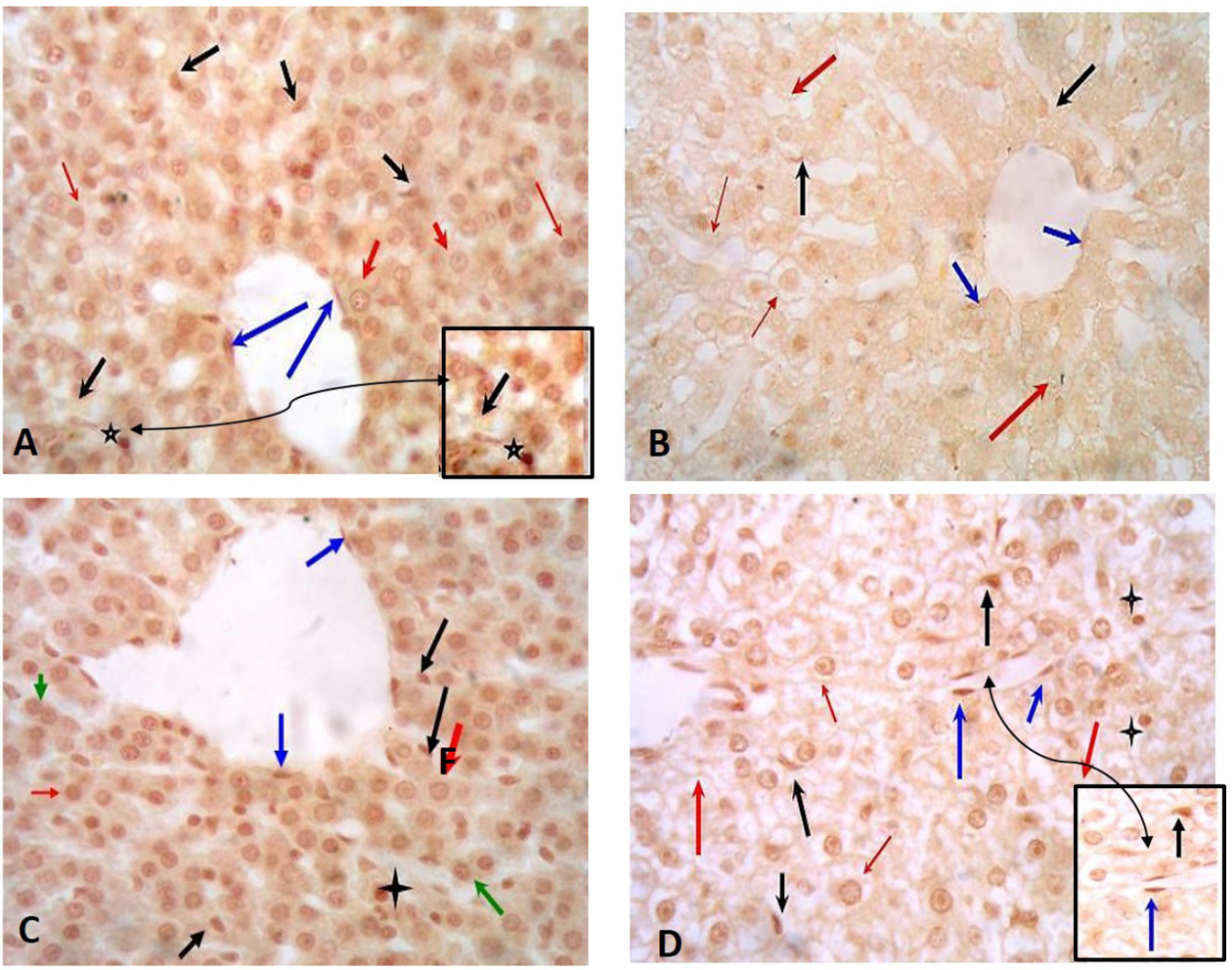


Fig. 4: Photomicrograph of the immunohistochemical localization of COX-1 in a rat liver tissue on:
A) The 7th postnatal day, showing the increase in the expression in all liver cells. Notice the higher cytoplasmic expression in the hepatocytes (red arrows). Notice that some hepatocytes show nuclear expression (thin red arrow) while others show perinuclear expression (thick red arrows). Notice also the high expression in the endothelial cells (blue arrows), Kupffer cells (black arrows), and the lymphocytes (stars).
B) The 14th postnatal day, showing the decline in the expression in the hepatocytes (red arrows), endothelial cells (blue arrows) and kupffer cells (black arrows). Notice that some hepatocytes show nuclear staining (thin red arrows), others failed to show this expression (thick red arrows).
C) The 21st postnatal day, showing the increase in the expression in all liver cells. The hepatocytes show high nuclear (thin red arrow), perinuclear (thick red arrow) and cytoplasmic expression. Notice the high expression in the hepatocytes (green arrows), endothelial cells (blue arrows), Kupffer cells (black arrows) and lymphocytes (star).
D) The 28th postnatal day, showing that the expression is mainly observed in the Kupffer cells (black arrows) and the endothelial cells (blue arrows). The hepatocytes show perinuclear and weak cytoplasmic expression (thin red arrows) while others failed to show nuclear expression (thick red arrows). Notice the expression of lymphocytes (stars). (X 400; insets X 1000).

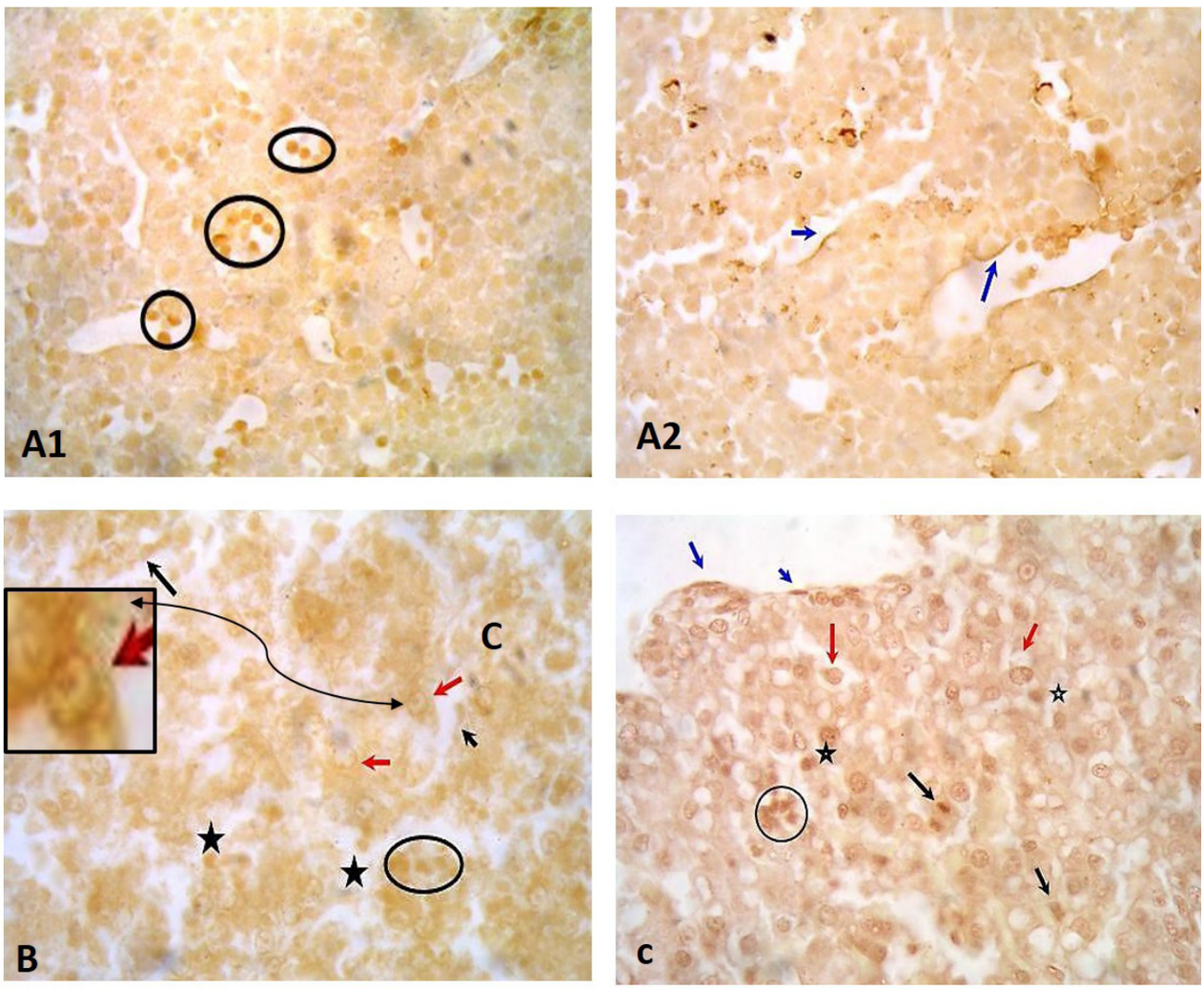


Fig. 5: Photomicrograph of the immunohistochemical localization of COX-2 in a rat liver tissue : A) the 18th day of prenatal life, showing A1) The expression in the immature hemopoietic cells (circle). Notice the cytoplasmic expression in the hepatocytes. A2) showing the expression in the endothelial cells (blue arrows) lining the central veins.
B) The 1st postnatal day, showing the patchy distribution of COX-2. Notice the decrease in the expression in the hemopoietic cells (encircled). The hepatocytes show the cytoplasmic and perinuclear zone expression (red arrows). Notice the little signals in the lymphocytes (stars) and in the Kupffer cells (black arrows).
C)The 7th postnatal day, showing the increase in the expression in the haemopoietic cells (encircled), hepatocytes (red arrows), the endothelial cells (blue arrows), the Kupffer cells (black arrows) and in the lymphocytes (stars). (X 400; insets X 1000).

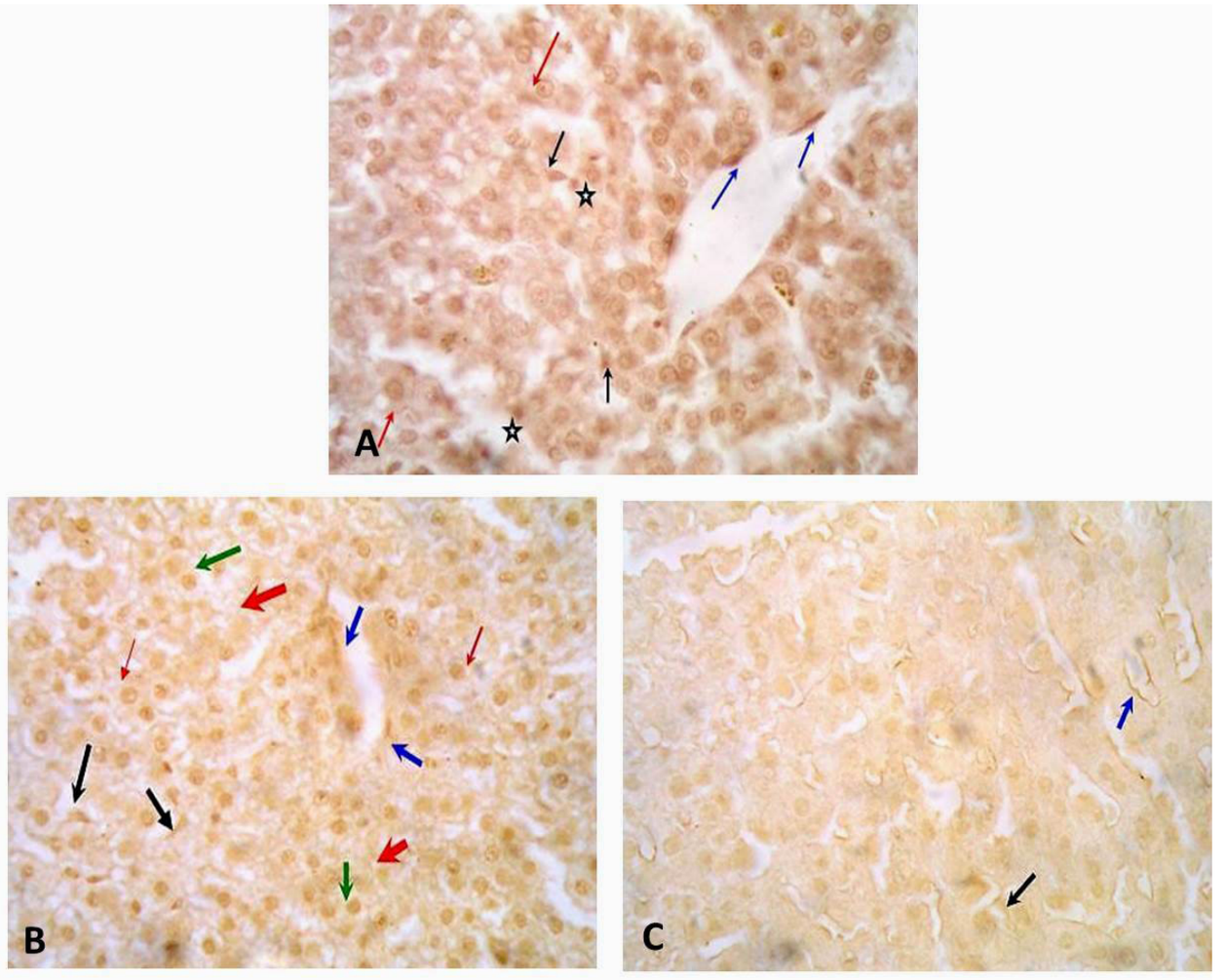


Fig. 6: Photomicrograph of the immunohistochemical localization of COX-2 in a rat liver tissue on:
A) The 14th postnatal day, showing that there is still high expression in the hepatocytes (red arrows), endothelial cells (blue arrows), Kupffer cells (black arrows), and the lymphocytes (stars).
B) The 21st postnatal day, showing the decrease in the expression in the hepatocytes (red arrows), Kupffer cells (black arrows), and hepatocytes (green arrow) and in the endothelial cells (blue arrows). Notice that some hepatocytes show nuclear signals (thin red arrow) while others fails to express COX-2. (thick red arrows).
C)The 28th postnatal day, showing that the expression is mainly localized to the endothelial cells (blue arrow) and Kupffer cells (black arrow). Magnification X100. (X 400).

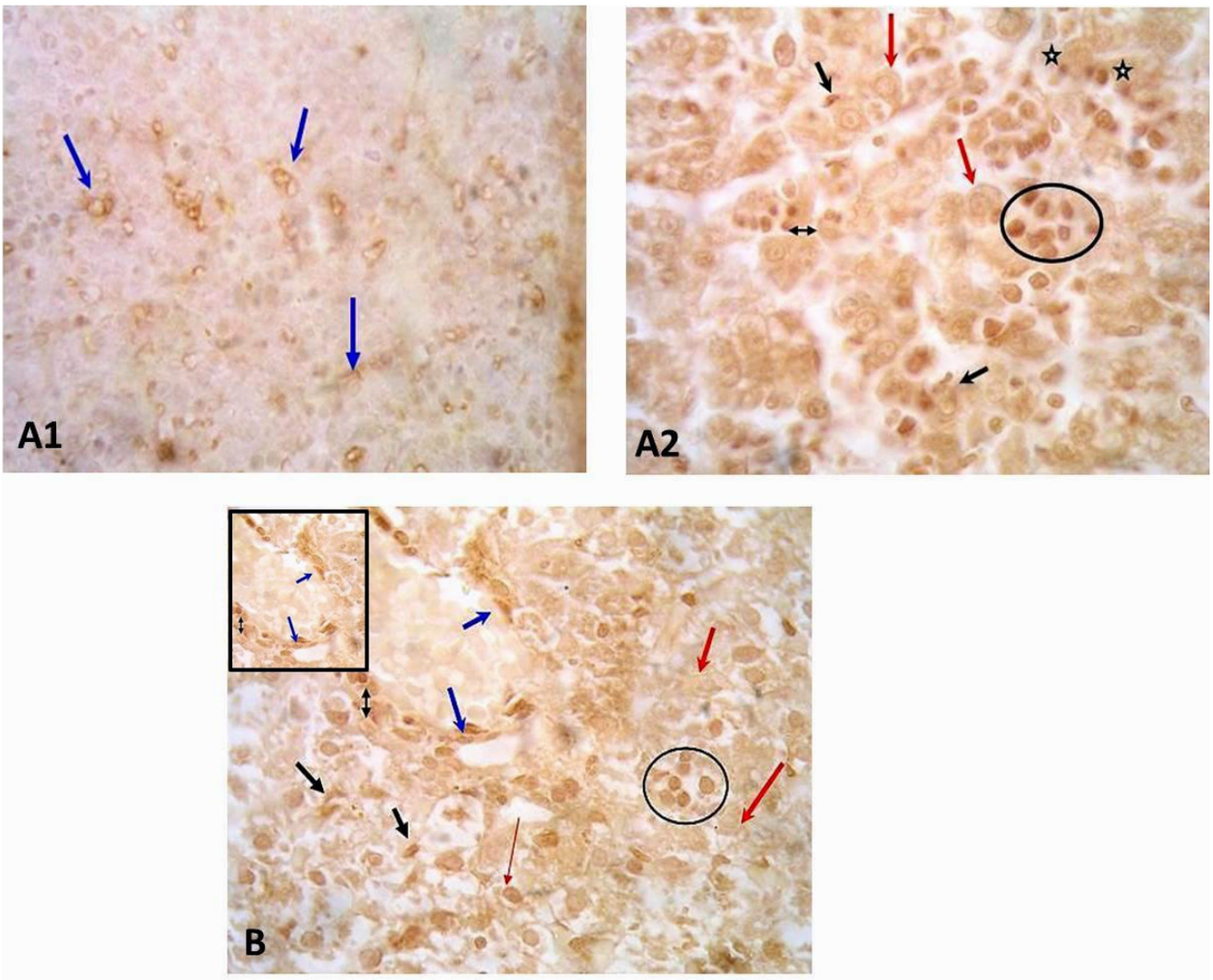


Fig. 7: Photomicrograph of the immunohistochemical localization of COX-3 in a rat liver tissue on:
A1) The 18th day of prenatal life, showing the COX-3 staining in the endothelial cells (blue arrows).
A2) The 1st postnatal day, showing high expression in the hemopoietic cells (circle) and high cytoplasmic and perinuclear expression in the hepatocytes (red arrows). Notice the high COX-3 signals the scattered lymphocytes (stars), the Kupffer cells (black arrows), and in the dividing lymphocytes (double arrow).
B) The 7th postnatal day, showing the nuclear expression in some hepatocytes (thin red arrow) whiles others showing no nuclear expression (thick red arrows). Notice the staining in the hemopoietic cells (encircled), endothelial cells (blue arrows) lining the central vein, Kupffer cells (black arrows) and in the dividing lymphocytes (double arrow). MagnificationX100**.**

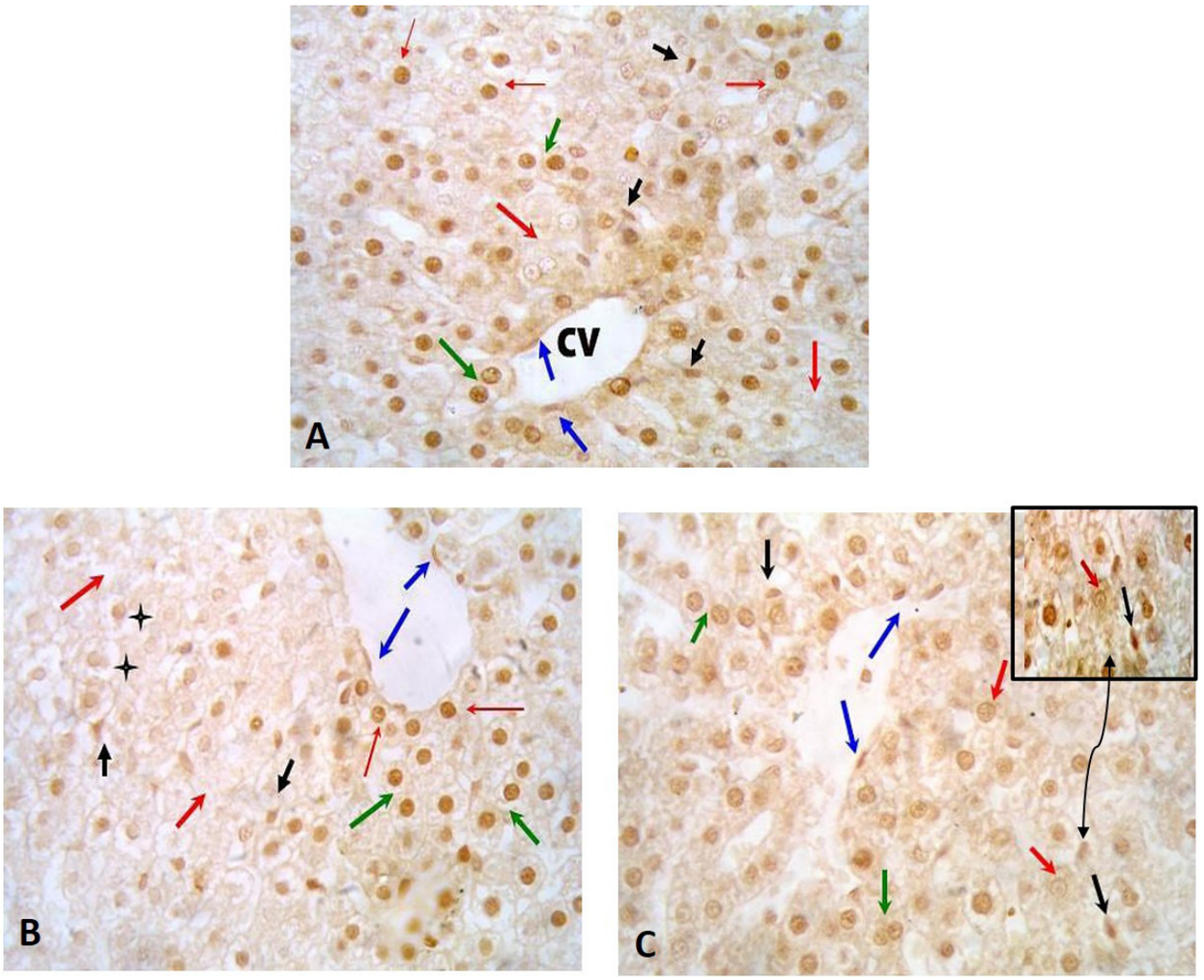
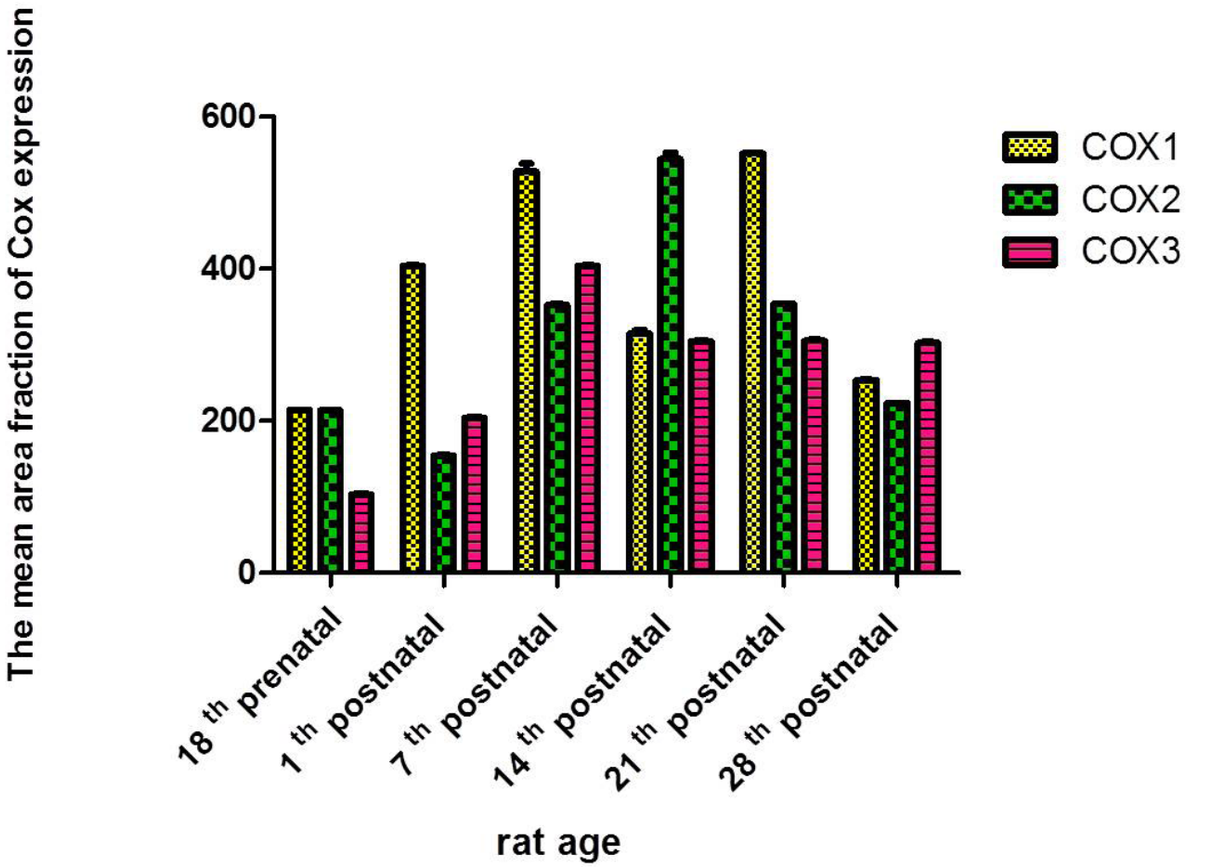


Fig. 8: Photomicrograph of the immunohistochemical localization of COX-3 in a rat liver tissue:
A) The 14th postnatal day, showing the high nuclear expression in the hepatocytes (thin red arrows), others show no nuclear expression (thick red arrows). Notice the expression in the dividing hepatocytes (green arrows), the Kupffer cells (black arrows), the endothelial cells (blue arrows) lining the central veins (CV). MagnificationX100.
B) The 21st postnatal day, showing the nuclear expression of the hepatocytes which are mainly localized in the centrilobular zone (thin red arrows), others show weak expression (stars) while others show no COX-3 signals (thick red arrows). Notice the high expression in the dividing hepatocytes (green arrows), the endothelial cells (blue arrows), and the Kupffer cells (black arrows).
C)The 28th postnatal day, showing the heterogeneity in the expression in the hepatocytes. The expression in the hepatocytes becomes mainly perinuclear (red arrows). Dividing hepatocytes (green arrows), endothelial (blue arrows), and Kupffer cells (black arrows) all are positive for COX-3 immunosignals. (X 400; insets X 1000).



Histogram I: The mean area fraction of the three types of COX in all animal groups. Results represent the mean ± SE (n = 8).

DISCUSSION

The COX isoforms; COX-1 and COX-2, had been reported to share more than 60% identity at the amino acid level. COX-1 is constitutively expressed in many tissues and is responsible for various physiological functions; including cytoprotecting of the stomach, vasodilatation in the kidney, and the production of a proaggregatory prostanoid, thromboxane A2[11]. In contrast, COX-2 is an inducible immediate early gene originally found to be induced by various stimuli such as mitogens and growth factors[12]. COX-3 is one of two recently identified splice variants of the COX- 1 gene and PCOX-1a in which part of intron 1 is, retained[13].

The liver has emerged as the major organ participating in the degradation and elimination of arachidonic acid products of systemic origin[6].

This study revealed that the expression of COX-1on the 18th day of prenatal life was mainly observed in the immature hemopoietic cells and the endothelial cells. Expression of COX-1 in the liver tissue at this age may help to explain the role of COX-1 in the liver tissue during its development and in the development of the hemopoietic cell. It was in accordance with West-Livingston *et al*., (2020) [14] who described that COX-1 mRNA was moderately abundant in embryos throughout organogenesis. It was also supported by Markmiller. (2010) who suggested that COX-1 mRNA was detected in the liver zebrafish during its development [15].

In the present research, it was noticed that COX-1 immunosignals were mainly observed in endothelial and Kupffer cells in all age groups. These findings were in line with the previous study[16] which reported that in normal liver, sinusoidal endothelial cells and Kupffer cells were the primary sources of COX-1 production. This was also in accordance with Kim *et al*. (2018) who found that COX-1 showed widespread staining along the sinusoid, suggesting that the positively labeled cells may be Kupffer cells and stellate cells[17].

In these results, COX-1 immunosignals were detected in the fetal and adult liver hepatocytes. This was in line with another previous work that reported that expression of COX-1 was occasionally to hepatocytes[3]. It suggested that in the hepatocytes COX-1 expression was weak in intensity

but was present in approximately 70% of hepatocytes and was distributed diffusely throughout the tissue. This was previously described by using double-immunofluorescence staining[18].

On the 14th postnatal day, COX-1expression in the hepatocytes showed the pattern of heterogeneity; some hepatocytes had shown nuclear expression while others failed to take this type of expression. This was in line with Ahmed. (2017) who found that COX-1 had distinct asymmetrical distribution especially evident in binuclear cells[19].

On the 28th postnatal day, these results revealed that the hepatocytes showed mainly perinuclear expression with some cytoplasmic expression. These findings are in accordance with Parfenova *et al*., (2001) they suggested that COX-1 localization in quiescent endothelial cells had two major sites of localization; the perinuclear zone (including the nuclear envelope) and the cytoplasm[20].

One of the controversial findings is the expression of COX-2 on the 18th day of prenatal life, in the present study COX-2 immunosignals were observed in the hepatocytes and this expression was completely absent on the 28th postnatal day. These were in agreement with another study that suggested that demonstrated COX-2 expression occur in fetal primary rat hepatocytes[21]. It was also in agreement with previous work that found only fetal hepatocytes, which exhibit a phenotype distinct from the adult counterpart, expressed COX-2[22]. Jonssen al., (2002) suggested that COX-2 staining was detected in the liver zebrafish during its development[23].

It was in contrast to the view of Bukiya (2019), they found that COX-2 mRNA was undetectable in rat embryos throughout organogenesis by any assay[24].

The expression of that enzyme in the fetal liver may explain its role in liver development. This was in line with a previous study that suggested the importance of COX-2 during the development[25]. It was also in agreement with other researchers who suggested that COX2 was involved in the regulation of cell growth angiogenesis[26]. Some researchers suggested that COX-2 expression had been associated with cell growth regulation[27].

on 7th postnatal day, COX-1 expression resembled the COX-2 in its distribution in the liver tissue. It was in accordance with previous work that found although the differences in the amino acid sequences, COX-1 and COX-2 might segregate into unique cellular compartments[20].

In this study, it was observed on the 14th postnatal day, during increasing in the expression of COX-2 in the liver cells, the COX-1 expression started to decline. This was supported by the previous results which showed that COX-2 up-regulation might be playing a role in COX-1 down regulation[21].

In the present research, COX-2 staining was observed in the dividing hepatocytes on the 21st postnatal day. This may explain the role of COX-2 in the hepatocyte's division and regeneration. This was with pervious work which observed that prostaglandin (PGs) produced by COX-2 were important for the early steps of liver regeneration[21].

The present results showed on the 21st postnatal day, the nuclear expression in some hepatocytes. The nuclear immunosignals in the hepatocytes may explain a possible physiological role of COX-2 in the nuclear functions in hepatic cells. This was in agreement with previous work that detected that COX-2 is involved in the regulation of nuclear functions[28]. It also suggested that COX-2 overexpression has been linked to the cell cycle progression and proliferation. They noticed that COX-2 nuclear and perichromatin zone localization sites and trafficking between the nucleus and cytoplasm in endothelial cells may indicate a novel function of COX-2 in regulating gene expression.

One of the controversial findings is the expression of COX-2 in the adult hepatocytes. In this study on the 28th postnatal day, it is obvious that COX-2 expression was mainly restricted to endothelial and Kupffer cells. These results were in line with Zidar *et al*., (2009) they observed that COX-2 is an inducible enzyme however, several tissues, including the liver, also express COX-2 constitutively[29]. These findings were also in agreement with other researchers, who stated that normal liver tissue had a low level of COX-2[30]. This was in contrast to the view of Chariyalerrttsa *et al*., (2001), they observed that endothelial and Kupffer cells were both negative for COX-2 protein[31].

This study revealed that the expression of COX-2 in the endothelial and Kupffer cells might help to explain the protective role of COX-2 in the liver tissue through production of PGs. This finding was in line with Wang *et al*., (2010), they noticed the protective effects of COX-2 within the liver were mediated through the production of PGE and PGI, which exert anti-inflammatory functions[32]. In our results on the 28th postnatal day, the hepatocytes were negative for COX-2. This result was in agreement with Chan *et al*, (2004) who demonstrated that COX-2 not expressed in normal adult primary rat or mouse hepatocytes.

In this work there was a trial to show the sites of COX-3 (the most recent detectable isoform of COX) in the liver tissue at the level of cellular localization.

COX-3 expression was noticed in fetal and adult liver tissue. It was in agreement with Chariyalertsak *et al*., (2002) who detected COX-3 mRNA in adult and fetal liver tissue by using Human Multiple Tissue Northern blots (MTN), while he did not detect it at the level of cellular localization[31].

On the 14th postnatal day, it was noticed the COX-3 expression in the hepatocytes mainly localized in the centrolobular zone (zone-3). This finding was in agreement with Humpton *et al*., 2019 who suggested that in the liver in zone -3 the cells are especially rich in enzymes involved in glycolysis and lipid and drug metabolism[33].

It was found that on the 28th postnatal day, COX-3 resembled COX-1 more than COX-2. This was in agreement with Chandrasekharan *et al*., (2002) they suggested that COX-3 possesses COX activity that differs pharmacologically from COX-1 and COX-2 but is more similar to COX-1[3].

COX-3 may be involved in the biosynthesis of endogenous anti-inflammatory mediators. It is speculated that such an enzyme may induce cyclopenetanone prostaglandins[34]

Taken together it could be concluded that the expression of COX isoforms in liver tissue plays an important role in the development and maturation of different cells population either through PG production or a novel gene production. Nuclear localization of COX in the hepatocytes arise a lot of questions about the role of COX in the enhancement of liver mitosis and maturation. This will open the future for a new rationale of the optimal therapeutic use of COX in liver diseases.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

التعبير التطويري للسيكلوأوكسجينيز فى نسيج كبد الفأر

ساره محمد نجيب عبد الحافظ ، سها عبد القوى ، سعديه رجب
قسم الأنسجة وبيولوجيا الخلية – كليه الطب جامعة المنيا

المقدمة: يوجد ثلاث انواع مختلفه للسيكلواوكسجسنيز بنسب مختلفة في الكبد وتتغير مع نمو الكبد.

طرق البحث: هذه الدراسة قامت على عينات أخذت من خلايا الكبد من ذكر فئران من الفصيلة (Sprague Dawley) وقد تم استخدام عدد (٨) أجنه من الفئران (١٨ يوم قبل الولادة) و(٤٠) فأر بعد الولادة باستخدام أعمار مختلفة , أما بالنسبة للأعمار التي استخدمت بعد الولادة, فهي كالاتي: (يوم , سبع سبع أيام ,اربع عشر يوما, واحد و عشرون يوما - ثمانية و عشرون يوما) التي تم تحضيرها لهذه ا لدراسة قد تم استخدام هذه الحيوانات بعد خضوعها للقتل الرحيم بواسطة استخدام مخدر الهالوسين و على الفور تم وضع عينات الكبد المستخرجة من هذه الفئران فالرمالين لمده ٢٤ ساعه.

وتناولت الدراسة الحالية دراسة مفصلة عن التعبير التطويري للسيكلوأوكسجينيز (٣,٢,١) فى خلايا الكبد باستخدام الطرق الهستوكيميائية.

النتائج: قد تمت الدراسة باستخدام المجهر الضوئي وأظهر تحليل النتائج ما يلي: كان ظهور الأنواع الثلاثه من السيكلوأوكسجينيز فى خلايا الكبد مختلفا. صبغة السيكلوأوكسجينيز بأنواعه الثلاثة كانت تتغير مع نمو خلايا الكبد. سيكلوأوكسجينيز(١) كان شبيها في بعض الأعماربالسيكلوأوكسجينيز(٢) ولكن وجد أنه أيضا شبيها في أعمار أخرى بالسيكلوأوكسجينيز(٣).

هذه النتائج السابقة تظهر دور السيكلوأوكسجينيز بأنواعه الثلاثة في نمو خلايا الكبد مما يفتح المجال فى المستقبل لاستخدام السيكلوأوكسجينيز بطريقة جديده ومتطورة في أمراض الكبد المختلفة.