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Protective effect of lycopene on celecoxib induced hepatotoxicity in albino rats: A histological study

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Abstract: Celecoxib, a selective COX-2 inhibitor, is an effective analgesic and anti-inflammatory drug. However it may cause adverse effects on liver, kidney and GIT. Lycopene is a natural antioxidant that may prevent oxidative injury in liver, kidney and GIT etc. Therefore this study is planned to observe the protective effect of lycopene on celecoxib induced hepatotoxicity. This experimental study was performed on the thirty adult male albino rats which were randomly divided into three groups, Group A served as control, Group B was treated with celecoxib 50 mg/kg and Group C was treated with Celecoxib 50 mg/kg along with lycopene 50 mg/kg for 30 days. At the end of the study, the animals were sacrificed and staining of liver tissue was done with hematoxylin and eosin. The results revealed swollen hepatocytes containing fat vacuoles and shrunken nuclei. Dilated hepatic sinusoids and congested central veins were also observed along with marked increase in the serum levels of alanine aminotransferase and alkaline phosphatase in the celecoxib treated group. These damaging effects were prevented and recovered to a greater extent in group receiving celecoxib along with lycopene. The present study has shown that the use of lycopene along with celecoxib protects against celecoxib induced toxicity in liver. Hence it can be recommended that the concomitant intake of lycopene as a supplement with celecoxib can prevent the hepatotoxic and other side effects of it.

Keywords: COX enzymes, celecoxib, hepatotoxicity, lycopene, antioxidant.

INTRODUCTION

Pain is one of the most common presenting complaints of a patient. It is generally associated with inflammation (Visha 2013).

NSAIDs (Non-steroidal Anti Inflammatory drugs) are used universally in both human and veterinary medicine whose efficacy has been proved in the treatment of inflammation and pain (Suleyman *et al*., 2007). They are commonly prescribed in fever, acute pain, rheumatoid disease, osteoarthritis, ankylosing spondylitis and menstrual cramps etc. They relieve the pain and inflammation of the disease but do not cure the disease (Somanath and Sowmaya, 2014).

It is scientifically proven that the therapeutic effects of NSAIDs depend primarily on cyclooxygenase (COX) enzyme inhibition. The main mediator link between pain and inflammation are prostaglandins (Visha 2013). Formation of prostaglandins from arachidonic acid is mainly regulated by COX enzyme inhibition (Suleyman *et al*., 2007). Arachidonic acid is released from phospholipid membrane by the action of phospholipase and thereafter metabolized either by lipooxygenase or cyclooxygenase pathway to produce leukotrienes (Visha, 2013).

Cyclooxygenase enzyme exists in two isoforms i.e. COX-1 and COX-2. COX-1 is a constitutive form that is present in most of the tissues under normal physiological conditions, whereas COX-2 is not normally detected in healthy tissues and its activity is induced by inflammatory stimuli. Thus, it enhances the synthesis of prostaglandins (Kockaya *et al*., 2010). COX-2 activation is exclusively increased during inflammatory processes and tissue injury (Schmeltzer *et al*., 2016).

It has been reported that NSAIDs can cause tissue damage due to oxidative stress that is induced by the production of free radicals (Bessone *et al*., 2016; Sozer *et al*., 2011). Evidence suggests that NSAIDs exert their toxic effects and cause tissue damage in GIT, liver and kidney via oxidative stress (Mahmood *et al*., 2009).

Celecoxib is a selective COX-2 inhibitor (Modi *et al*., 2012). It is one of the most widely prescribed and used COX-2 inhibitors (Suleyman *et al*., 2007). Celecoxib may cause liver damage, allergic reactions and nephrotoxicity (Boelsterli *et al*., 2013; Mukthinuthalapati *et al*., 2018).

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Natural antioxidants are the recent source of protection which may prevent free radical cell damage. Several dietary antioxidants prevent oxidative damage such as lycopene, sulforaphane, and vitamin C. Lycopene is a naturally occurring antioxidant. It is the most effective and abundant of all the carotenoids present in human tissues as well as in human blood. Lycopene is the most widely and commonly used carotenoid in daily dietary food intake and is proved to be a potent antioxidant (Waer and Shalaby, 2012). Tomatoes, water-melons, pink grape-fruits and pink guavas are the rich sources of lycopene (Pal and Gowda, 2014). It is a strong antioxidant and this property explains its health promoting effects (Ni *et al*., 2019). Since it can trap singlet oxygen and free radicals, that's why it acts as a potent antioxidant (Pal and Gowda, 2014). Lycopene minimizes the risk of some chronic diseases like liver dysfunctions, cardiovascular diseases, gastropathy etc. and has been shown to reduce tumor cell hyperplasia & cellular DNA injuries (Liang *et al*., 2012). Pina-Zentella *et al*., (2016) explain that the intake of lycopene inhibits oxidative injury i.e. lipid peroxidation in the liver.

In the light of above account, since no histological study has been carried out until now to explain the protective role of lycopene on celecoxib treated liver, so this option has been availed to undertake this research.

MATERIALS AND METHODS

This experimental study was performed in the animal house and research laboratory of the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, for 30 days. All procedures performed in the study were in accordance with Helsinki declaration (1964) and study was approved by the institution's ethical committee with Letter No.F.1-2/BMSI-E.COMT/JPMC (Carlson *et al*., 2004).

Thirty healthy, young adult male albino rats, 90-120 days of age, weighing 190-210 gms were obtained for this experimental study. The animals were handled as per guidelines of US National Research Council's Guide for the Care and Use of Laboratory Animals. They were acclimatized to the environment one week prior to the commencement of the study and their health status and dietary intake was observed (The Guide, 2011). After one week they were treated with celecoxib (GETZ Pharma), 50 mg/kg and lycopene (General Nutrition Corporation), 50 mg/kg, according to the experimental dose (Koackaya *et al*., 2010; Bignotto *et al*., 2009).

The experimental animals were divided into three main groups:
Group A Control group was kept on standard laboratory diet and water ad libitum.
Group B was given Celecoxib 50 mg/kg orally once daily.
Group C was given Celecoxib 50 mg/kg orally along with Lycopene 50 mg/kg orally once daily.

All the animals were weighed and housed in cages, in a room maintained with a 12-hour dark and light cycle under laboratory environment. They were given standard laboratory diet and water ad libitum (The Guide, 2011). All the animals were slaughtered at the end of their treatment episode. A median longitudinal incision was given and abdominal viscera were carefully exposed. Gross appearance of liver was noted and then liver was detached from the body of the animal and weighed. Liver was divided into two halves from the plane dividing it into left and right lobes. Both the halves were then fixed in buffered neutral formalin for 24 hours. Thereafter the tissue was dehydrated via passing through ascending strengths of alcohol (70%, 80%, and 95%) for an interval of one hour in each solution followed by absolute (100%) alcohol l and ll, one hour each. Then the tissue was passed through xylene I and II each for an hour, thus making it accessible to the infiltrating/embedding medium. Then the tissue was embedded in paraffin at 59 degree centigrade in tissue embedding system. 4-5 micron thick sections of hepatic tissue were stained with hematoxylin and eosin (Bancroft and Gamble, 2008). Then the sections were observed at 40 X objectives of light microscope and results were made.

STATISTICAL ANALYSIS

The statistical significance of the differences of quantitative data between treated and control rats was calculated by the Student's t-test. Significance of P-value is taken as: $P>0.05$* (Non-significant), $P<0.05$** (Significant), $P<0.005$*** (Moderately significant), $P<0.001$**** (Highly significant). SPSS version 20 was used for this purpose.

RESULTS

At the of complete experimentation, microscopic examination of H & E stained sections of control animals (Group A) showed normal organization of liver parenchyma, composed of cords of hepatocytes radiating from the central vein; separated from each other by hepatic sinusoids. The hepatic lobules appear almost hexagonal in shape. Polygonal hepatocytes appeared normal with distinct boundaries and eosinophilic cytoplasm. Nuclei were rounded and centrally placed with 1-2 nucleoli as illustrated by fig. 1. Portal and periportal areas were seen normal as shown in fig. 2.

Celecoxib treated (Group B) tissue sections showed disorganized architecture of hepatic lobules with sinusoidal dilatations. Hepatocytes were ballooned up and vacuolated with pyknotic nuclei. Dilatation and

congestion of central vein was noted as illustrated by fig. 3. Portal areas were dilated with mononuclear infiltration as seen in fig. 4.

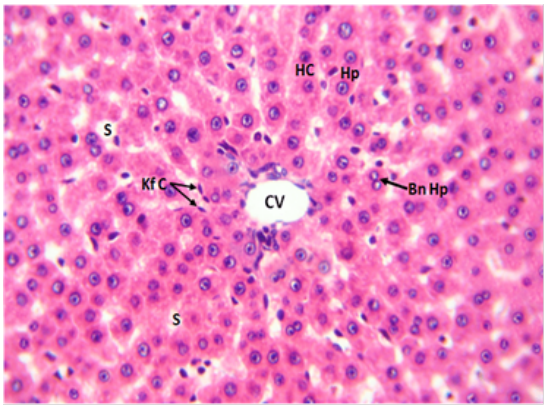

Fig. 1: H & E stained, 4 µm thick liver tissue section of Group A animal illustrating central vein (CV), sinusoids (S), hepatocytes (Hp) organized in the form of hepatic cords (HC), binucleate hepatocytes (BnHp) and Kupffer cells (Kf C). (Photomicrograph x 400)

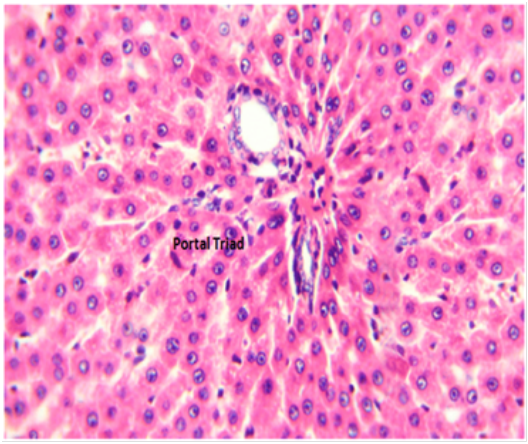

Fig. 2: H & E stained, 4 µm thick liver tissue section of Group A animal illustrating Portal Triad including hepatic artery, portal vein and bile ductule. (Photomicrograph x 400)

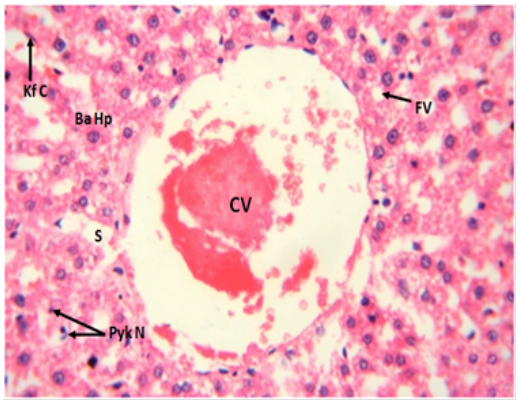

Fig. 3: H & E stained, 4 µm thick liver tissue section of Group B animal illustrating dilated and congested central vein (CV) with hemorrhage, ballooned hepatocytes (Ba Hp) along with fat vacuole (FV), pyknotic nuclei (Pyk N), dilated sinusoids (S) and Kupffer cells (Kf C). (Photomicrograph x 400)

Lycopene protected (Group C) showed restoration of almost normal architecture of hepatic lobules. Hepatocytes were seen normal and polygonal with lesser pyknotic nuclei. Central veins were slightly dilated but not congested. Sinusoids appeared almost normal as explained by Fig. 5. Roughly normal portal areas were seen with slight mononuclear infiltration can be observed in fig. 6.

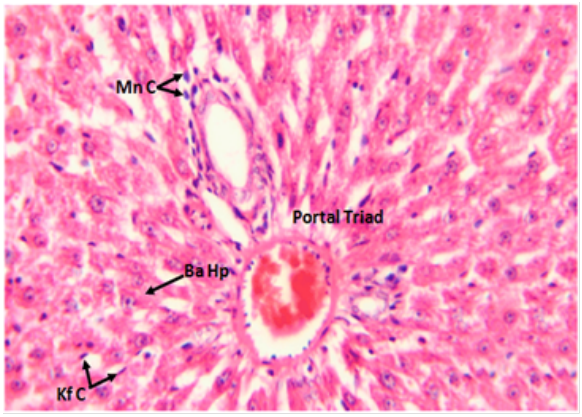

Fig. 4: H & E stained, 4 µm thick liver tissue section of Group B animal illustrating dilated and distorted Portal Triad, ballooned hepatocytes (Ba Hp), pyknotic nuclei (Pyk N) and mononuclear cell infiltration (Mn C). (Photomicrograph x 400)

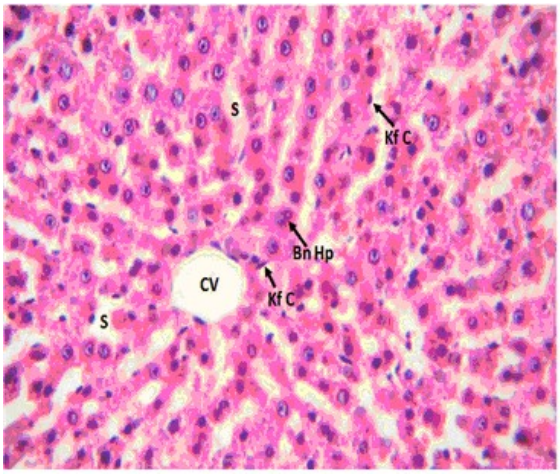

Fig. 5: H & E stained, 4 µm thick liver tissue section of Group C animal illustrating central vein (CV), binucleate hepatocytes (BnHp) and slightly dilated sinusoids (S) along with kupffer cells (Kf C). (Photomicrograph x 400)

Statistical analysis of the difference in the mean level of serum ALT in different treated groups of albino rats showed a moderately significant increase ($P<0.005^{***}$) in the enzyme level of group-B in comparison to control

group-A (table 1). Whereas there was a highly significant decrease ($P<0.001^{****}$) observed in serum ALT level of group-C animals when compared to group-B (table 1).

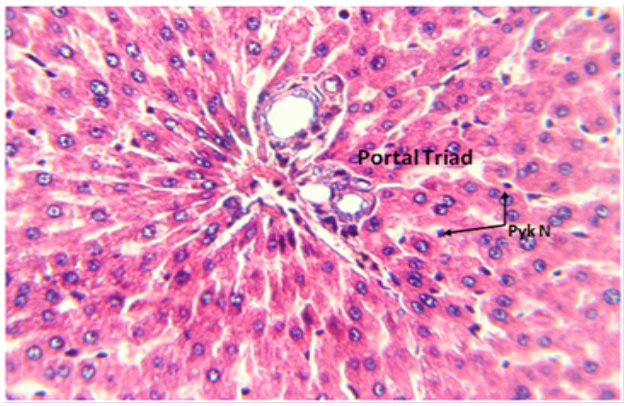

Fig. 6: H & E stained, 4 µm thick liver tissue section of Group C animal illustrating slightly dilated Portal Triad, hepatocytes and few pyknotic nuclei (Pyk N). (Photomicrograph x 400)

Table 1: Mean* level of serum ALT (IU/L) in treated groups of albino rats

Groups	Treatment given	Serum enzyme level-ALT
A (n=10)	ND	75.0±3.16
B (n=10)	Celecoxib	90.4±1.14
C (n=10)	Celecoxib + Lycopene	80.0±1.58

*Mean±SEM

Statistical analysis of the difference in the mean level of serum Alanine Aminotransferase in different groups of Albino rats.

Statistical comparison	P-value
B vs. A	$P<0.005^{***}$
C vs. A	$P<0.05^{**}$
C vs. B	$P<0.001^{****}$

Statistical analysis of the difference in the mean level of serum ALP in different treated groups of albino rats revealed a highly significant increase ($P<0.001^{****}$) in the serum ALP level of group-B as compared to control group-A (Table-2).Whereas there was a highly significant decrease ($P<0.001^{****}$) observed in serum ALP levels of group-C in comparison to group-B (table 2).

Table 2: Mean* level of serum ALP (IU/L) in treated groups of albino rats

Groups	Treatment given	Serum enzyme level-ALP
A (n=10)	ND	95.0±1.58
B (n=10)	Celecoxib	110.40±1.14
C (n=10)	Celecoxib + Lycopene	100.0±1.58

*Mean±SEM

Statistical analysis of the difference in the mean level of serum Alkaline Phosphatase in different groups of Albino rats.

Statistical comparison	P-value
B vs. A	P<0.001****
C vs. A	P<0.05**
C vs. B	P<0.001****

Key: ALT…Alanine Aminotransferase; ALP...Alkaline Phosphatase; IU/L...International Unit/Liter; ND...Normal diet; Non-significant*; Signifcant**; Moderately significant***; Highly significant****; SEM…Standard Error Mean; <…less than; >…greater than; vs…versus

DISCUSSION

NSAIDs are one of the most frequently prescribed drugs worldwide. They are primarily utilized owing to their analgesic, anti-inflammatory and antipyretic properties (Somanath and Sowmaya, 2014). Due to the regular use of these anti-inflammatory and analgesic agents, their toxic effects do arise mostly in GIT, liver or kidney particularly when they are used to alleviate pain, fever or inflammation in higher doses and for a lengthy duration. Celecoxib, a specific COX-2 enzyme inhibitor, is extensively used as an analgesic and anti-inflammatory remedy in rheumatoid and osteoarthritis. It is proven to cause cholestatic and hepatocellular injury (Modi *et al*., 2012).

Proper antioxidant may offer protection against free radical damage and oxidative stress. Lycopene is a naturally existing dietary carotenoid which is found to exhibit antioxidant, anti-inflammatory and anti-carcinogenic activity in liver and other organs. It is revealed that lycopene minimizes oxidative injury in liver (Waer and Shalaby, 2012).

In the present study, microscopic examination of hematoxylin and eosin stained liver tissue sections of celecoxib treated group-B animals revealed distortion of liver parenchyma with fatty infiltration which induced microvesicular steatosis and vacuolation of hepatocytes,

ballooning and necrotic changes of the hepatocytes with the small pyknotic nuclei. Sinusoids and vessels were markedly dilated and congested. These findings were caused by celecoxib as a result of increase lipid peroxidation and lipogenesis, it also promoted oxidative injury to the hepatocytes as explained by Ni and friends (2019). It is also previously described that impaired electron transport chain by COX-2 inhibitors caused inhibition of oxidative phosphorylation in the mitochondria of the hepatocytes that leads to the accumulation of reactive oxygen species which ultimately causes the mitochondrial dysfunction and cell death (Maharjan *et al*., 2014). The same findings were found by Kockaya and friends (2010), who observed the pathological and biochemical effects of celecoxib in wistar albino rats.

Lycopene recovered the celecoxib induced oxidative damage to the liver when it was given along with celecoxib to the group-C animals. Liver parenchyma restored its cytoarchitecture near to normal. This was attributable to the antioxidative and anti-hyperlipidemic properties of lycopene which prohibited oxidative phosphorylation, hyperlipidemia and oxidative injury to the cell. These findings were in accordance with Waer and Shalaby (2012) who studied the protective effects of lycopene on hepatotoxicity induced by gamma radiation in rodents.

The levels of serum ALT and ALP were found to be raised in celecoxib treated animals. This was due to the celecoxib induced oxidative damage to the hepatocytes which increased the leakage of hepatic enzymes from the cytoplasm of hepatocytes into the circulation, as suggested by Niranjan and friends (2010). This was also in accordance to Schmeltzer (2019) who observed that the hepatotoxic effects of COX-2 inhibitors raised the levels of serum hepatic enzymes.

A moderate decrease in serum hepatic enzyme levels was noticed when lycopene was given along with the celecoxib to the group-C animals. This was probably caused by the protective effect of lycopene against free radical cellular damage as explained previously by El-Sayed *et al*. (2015) who noticed a reduction in the serum liver enzyme levels after giving lycopene in carbon tetrachloride induced hepatocellular damage.

CONCLUSION

On the basis of the above-mentioned study, it is concluded that celecoxib induces liver damage and lycopene ameliorates the hepatic damage induced by celecoxib. Hence it can be recommended that the concomitant use of lycopene as a supplement or as a dietary product with celecoxib can prevent the hepatotoxic and other side effects of it.

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