The PI3K/Akt pathway in colitis associated colon cancer and its chemoprevention with celecoxib, a Cox-2 selective inhibitor

NSAIDs are known to exert anti-tumor effects in various organ systems including several human cancer cell lines and animal models (Soh and Weinstein, 2003). NSAIDs act primarily by the inhibition of Cox enzyme, thereby inhibiting the synthesis of prostaglandins (Gurpinar et al.; 2014). The involvement of COX-2 in tumorigenesis and its overexpression in various cancer tissues suggest that inhibition of COX-2 is responsible for the chemopreventive efficacy of these agents (Gurpinar et al., 2014). However, emerging evidences also suggest for the Cox-independent effects of NSAIDs in case of the inhibition of tumorigenesis (Grösch et al., 2006). These involve the modulation of several important signal transduction pathways, where PI3K/Akt/PTEN pathway is a prominent area of our interest.

Fig. 4.6.1: Schematic representation of the PI3K-AKT signaling pathway (Meier et al., 2005).

The phosphatidylinositol 3-kinase (PI3K) pathway is critical for cell survival and cell growth, and can be activated by growth factors binding to cell surface receptors (Fig. 4.6.1). PI3Ks are a family of related intracellular signal transducer enzymes capable of phosphorylating the 3’ position hydroxyl group of the inositol ring of phosphatidylinositol (Fruman et al., 1998; Courtney et al., 2010). PI3K pathway is an intricate signalling cascade that is among the
most frequently activated pathways in cancer (http://www.biooncology.com/therapeutic-targets/pi3k). Several studies have suggested that the stimulation of a variety of receptor tyrosine kinases and G-protein coupled receptors result in PI3K activation, which further leads to the production of phosphatidylinositol- 3,4,5-trisphosphate (PIP3) from phosphatidylinositol-4,5-bisphosphate (PIP2). (Fresno et al., 2004). PIP3 is a second messenger which generates a cascade of downstream signalling molecules. PIP3 does not activate PKB/Akt directly but instead appears to recruit PKB/Akt to the plasma membrane and to alter its conformation to allow subsequent phosphorylation by the phosphoinositide-dependent kinase-1 (PDK1) (Nicholson and Anderson, 2002). Serine-threonine protein kinase AKT (also known as protein kinase B) is a major downstream target of PI3K for regulating tumor growth and angiogenesis (Okumura et al., 2012). Further, PTEN is a dual specificity phosphatase that dephosphorylates phosphatidylinositol 3,4,5-trisphosphate to phosphatidylinositol-4,5-bisphosphate, and thus, is a potent antagonist of phosphoinositide-3-kinase (PI3K)/ATP-dependent tyrosine kinases (Akt) signalling (Stambolic and Suzuki, 1998). The active form of Akt (p-Akt) phosphorylates and inactivates the tumor suppressor kinase, GSK3β. Now β-catenin, which otherwise stays in an inactive form by the action of GSK3β, moves to the nucleus and leads to the transcription of proteins involved in the cell proliferation and invasion (Broek et al., 2013).

**Fig. 4.6.2:** Schematic representation of the PI3K-AKT-PTEN signaling pathway and its correlation with angiogenesis. (Okumura et al., 2012).
The catalytic subunit of PI3K, as well as the protein called the Phosphatase and Tensin homolog tumor suppressor (PTEN) a negative regulator of this pathway, are commonly mutated in a wide range of human tumors (Knight and Shokat, 2007). Genetic alterations of PI3K lead to the dysfunction of vasculature and angiogenesis (Fig. 4.6.2). In addition, forced expression of PI3K alone is sufficient to increase angiogenesis via increased VEGF expression (Skinner et al., 2004). Both the PI3K and angiogenic pathways play an important role in the growth and development of tumors, and we presently explored their role in ulcerative colitis mediated colon carcinogenesis.

The interwoven oncogenic and angiogenic pathways

The oncogenic PI3K and the angiogenic Vegf pathways are known to regulate each other in case of leukemia. Both of these pathways are thought to be regulated by tyrosine kinases, presently Jak-3 regulating the PI3K and Vegf regulating the angiogenic pathways. We found an increased expression of both these tyrosine kinases as studied by western blots as well as immunofluorescent analysis in DSS, DMH and DSS+DMH groups (Fig. 4.6.3, 4.6.4a-b, 4.6.10a-b). The co-administration of celecoxib in these groups brought their levels back towards normal. Also, major components of the PI3K and angiogenic pathways were altered including PI3K, p-Akt, PTEN, PDK1, Wnt, β-catenin, Vegf, MMP-2, MMP-9 and iNOS in inflammatory as well as carcinogenic milieu. The protein expressions of PI3K, p-Akt, PDK1, Wnt, β-catenin, Vegf, MMP-2, MMP-9 and iNOS were found to be elevated in the groups treated with DSS, DMH and DSS+DMH, while the co-treatment with celecoxib in these groups lowered the levels of expression of these proteins (Fig. 4.6.3, 4.6.5a-b, 4.6.6a-b, 4.6.7a-b, 4.6.9a-b, 4.6.10a-b, 4.6.11a-b and 4.6.12a-b). The expression of PTEN, the negative regulator of this pathway was suppressed in the inflammatory and carcinogenic conditions while it was reverted towards normal with the administration of celecoxib in these groups (Fig. 4.6.8a-b).
Fig. 4.6.3: Shows protein expression of PI3K, PDK1, Wnt and β-catenin as studied by western blots. All these protein expressions were upregulated in DSS, DMH and their combinatorial group. These effects were reduced significantly by the co-administration of celecoxib. Also, the densitometric analysis of these blots showed similar results in the eight treatment groups, respectively (a, b, c, d, e). Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
Every major node of the PI3K pathway is frequently mutated or amplified in a wide variety of solid tumors (Yuan et al., 2008). Also, PI3K/AKT/PTEN pathway is important in the regulation of angiogenesis mediated by Vegf and Vegfr2 in many tumors including leukemia (Okumura et al., 2012). PI3K is a member of lipid kinase family characterized by their ability to phosphorylate PIP2 to PIP3, the latter being a second messenger in the downstream signal transduction cascade. RTK activation results in PIP3 production by PI3K at the inner side of the plasma membrane. There is interaction of these phospholipids with Akt, translocating it to the plasma membrane, where it is phosphorylated and hence activated by PDK1. p-Akt modulates the function of numerous substrates involved in the regulation of cell survival, cell cycle progression and cellular growth (Vander Haar et al., 2007). mTOR is found to have the ability to upregulate the PI3K/Akt pathway (Hemmings et al., 2012). PTEN, the negative regulator of this PI3K pathway, is illustrated by its frequent disruption in cancer. Its lipid phosphatase activity suppresses the PI3K/Akt/mTOR pathway (Song et al., 2012). It is localized on chromosome 10q23 that is often lost in late-stage human cancers, especially that of the prostate, brain, and endometrium (Tao et al, 2006). We presently investigated the role of PTEN and the other components of PI3K pathway in ulcerative colitis associated colon carcinogenesis.

Fig. 4.6.4a:
**Fig. 4.6.4b:**

Fig. 4.6.4b: Shows the protein expression of Jak-3 in different groups after the fluorescent immunohistochemistry of paraffin embedded tissue sections. This protein was overexpressed in DSS, DMH and DSS+DMH groups while it was suppressed with the administration of celecoxib in these groups. 

**Fig. 4.6.4a:** Shows the protein expression of Jak-3 in different groups after the fluorescent immunohistochemistry of paraffin embedded tissue sections. This protein was overexpressed in DSS, DMH and DSS+DMH groups while it was suppressed with the administration of celecoxib in these groups. b: Shows the immunofluorescent staining scores for Jak-3 in the form of stock chart (a, b, c, d: Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).

**Fig. 4.6.5a:**

Fig. 4.6.5a:
**Fig. 4.6.5a:** The photomicrograph shows the protein expression of p-Akt as studied by immunofluorescent staining. Increased expression was observed in the DSS, DMH and DSS+DMH groups, while it was reverted towards normal with the co-administration of celecoxib in these groups. 

**b:** Shows the quantitative analysis of the immunofluorescence of p-Akt (a, b, c, d) means in each group not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01.

**Fig. 4.6.6a:**

- **CONTROL**
- **DSS**
- **DMH**
- **C**
- **DSS+DMH**
- **DSS+C**
- **DMH+C**
- **D+D+C**
Fig. 4.6.6b: Increased protein expression of Wnt is seen in the DSS, DMH and DSS+DMH groups, which has been corrected with the co-administration of celecoxib in these groups. b: Shows the quantitative analysis of the immunofluorescence of Wnt and similar results are seen here (\textsuperscript{a, b, c} Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): \( p < 0.01 \)).

Fig. 4.6.7a:
Fig. 4.6.7b: The photomicrograph shows the upregulation of the expression of β-catenin in the DSS, DMH and DSS+DMH groups while this expression was reduced with the administration of celecoxib in these groups. b: The quantitative analysis of this immunofluorescent expression further supported these results where maximum score is seen for DMH, DMH+DSS and DSS groups, while it was reverted towards normal with the co-administration of celecoxib (a,b,c Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).

Fig. 4.6.8a:
Fig. 4.6.8b: The photomicrograph represents the protein expression of the tumor suppressor PTEN in different groups as studied by immunoflorescence and decreased expression of PTEN is visualized in the DSS, DMH and DSS+DMH groups while these were brought towards normal with the co-administration of celoxib in these groups.

Fig. 4.6.8a: The photomicrograph represents the protein expression of the tumor suppressor PTEN in different groups as studied by immunoflorescence and decreased expression of PTEN is visualized in the DSS, DMH and DSS+DMH groups while these were brought towards normal with the co-administration of celoxib in these groups. b: The quantitative analysis of this expression further supports these results (a, b, c, d Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).

Fig. 4.6.9a:
Three-dimensional tumor growth is dependent on the perpetual recruitment of host blood vessels to the tumor site (Rak et al., 2000). VEGF plays a key role in the endothelial cell differentiation and the process of angiogenesis. Transition from an orderly cellular arrangement to a three-dimensionally unrestricted "invasive" growth pattern relies on the collective ability of cancer cells to secure a non-interrupted access to host-derived blood vessels (Folkman, 1990). Angiogenesis is the key player in various pathologies including inflammation and cancer. VEGF expression in endothelial cells is mediated by the PI3K signalling (Jiang et al., 2000). VEGF binds to its receptor-2 (Vegfr2), an RTK, and subsequently activates the PI3K pathway by its phosphorylation (Ferrara, 2000). Oncogenes and tumor suppressor genes (PI3K/PTEN) not only regulate the expression of VEGF but also have their cumulative effect on tumor angiogenesis (Volpert et al., 1997). Angiogenesis plays a critical role in tumor invasion and metastasis. Therefore, an anti-angiogenic therapy is an attractive strategy for anti-cancer treatment (Song et al., 2012).
Fig. 4.6.10a: The photomicrograph represents the protein expression of VEGF which has been elevated in the inflammatory as well as carcinogenic conditions but this expression was reduced by the administration of celecoxib in these groups.

Fig. 4.6.10b: Shows the immunofluorescent staining scores of the different treatment groups. Means in each segment not sharing a common superscript letter that differed significantly (Duncan’s test): $p < 0.01$.
**Fig. 4.6.11a**: The photomicrograph represents the increased expression of MMP-9 in the DSS, DMH and DSS+DMH groups while the treatment with celecoxib has reduced these effects. **b**: Shows the stock chart of the immunofluorescent staining scores for MMP-9 by the quantitative analysis of the fluorescent expression of MMP-9 (\(a, b, c\) Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
Fig. 4.6.12: The photomicrograph shows the western immunobots of Vegf, MMP-2 and MMP-9. Increased expression of these agents of angiogenesis was observed in the DSS, DMH and DSS+DMH groups. The treatment with celecoxib lowered the levels of these proteins. The densitometric analysis of these groups showed similar results where i) to viii) represents the eight treatment groups, respectively (a, b, c, d, e Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).

We presently explored the protein expressions of VEGF, MMP-2 and MMP-9 by the immunofluorescent analysis and western blots and found their levels to be increased in the inflammatory as well as carcinogenic conditions. The co-administration of celecoxib in these groups decreased the expression levels of these proteins towards normal (Fig. 4.6.10-4.6.12).
Fig. 4.6.13: The photomicrograph shows the gelatine zymography of the different treatment groups where the differential activity of the matrix metalloproteinases have been represented. Higher activity of the activated and pro-forms (zymogens) is seen in the DSS, DMH and the DMH + DSS groups, suggesting their role in the degradation of extra cellular matrix (ECM) during angiogenesis, while it is decreased with the co-administration of celecoxib in these groups.

Gelatin zymography was done to study the relative activities of the pro- and active forms of MMP-2 and MMP-9. In the DMH and DSS treated groups, an increased activity of both pro- and active forms of MMPs was seen as compared to the control while these levels were reverted towards normal by the co-administration of celecoxib in these groups (Fig. 4.6.13).
Fig. 4.6.14a: The photomicrograph represents the increased expression of MIP-1 in the DSS, DMH and DSS+DMH groups while the treatment with celecoxib has reduced these effects. b: Shows the stock chart of the immunofluorescent staining scores for MIP-1 by the quantitative analysis of the fluorescent expression of MIP-1 (a, b, c, d). Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
Fig. 4.6.15a: The photomicrograph represents the increased expression of MCP-1 in the DSS, DMH and DSS+DMH groups while the treatment with celecoxib has reduced these effects.  

b: Shows the stock chart of the immunofluorescent staining scores for MCP-1 by the quantitative analysis of the fluorescent expression of MCP-1 (a, b, cMeans in each segment not sharing a common superscript letter that differed significantly (Duncan’s test): p < 0.01).
Chemokines are small cell attractant proteins that play an important role in several pathophysiological processes such as inflammation and immunity. Many proinflammatory chemokines also support the development of vascular blood supply at the site of inflammation (Salcedo and Oppenheim, 2003). The main function of CC subfamily of chemokines is considered to mediate and direct the trafficking and migration of monocytes and lymphocytes (Santoni et al., 2014). Stamatovic et al. observed that CCL2 type chemokines played a crucial role in mediating hemangioma growth and angiogenesis (Stamatovic et al., 2006). Moreover, treatment of immunodeficient mice bearing human breast carcinoma cells with a neutralizing antibody to MCP-1 resulted in significant increases in survival and inhibition of the growth of lung micrometastases (Salcedo et al., 2000). Thus, several of the CC chemokines are potent promoters of angiogenesis (Santoni et al., 2014).

We presently explored the role of two chemokines: MCP-1 and MIP-1 and found their levels to be elevated in the DSS, DMH and DSS + DMH groups while the co-administration of celecoxib in these groups brought their levels towards normal (Fig. 4.6.14a-b and 4.6.15a-b).

NO plays a central role in the angiogenic cascade by demonstrating that Vegf requires a functioning NO/cyclic guanosine monophosphate (cGMP) pathway within the endothelial compartment to promote neovascular growth. The role of reactive oxygen and nitrogen (RONS) species was studied and found to be elevated in DSS, DMH and DMH+DSS groups. These effects were corrected significantly by the administration of celecoxib in these groups (Fig. 4.6.16).

An increase in NOS activity is found in head and neck cancer (Gallo et al., 1998). The importance of NO in angiogenesis has been demonstrated in mice lacking or overexpressing the endothelial isoform of NOS (eNOS) (Amano et al, 2003). Oxidative stress releases RONS species, which are known to mediate many physiological and pathological functions, including angiogenesis and vascular permeability (Fukumura et al, 2001). Among the non-prostaglandin pathways, the free radicals have been implicated in cell signalling processes, called redox signalling. Moderately elevated reactive oxygen species (ROS) can also initiate redox signalling in a molecular cross talk with the targets, such as COX-2, NF-κB and iNOS, and may lead to an upregulation of angiogenesis (Setia and Sanyal, 2012).
Fig. 4.6.16: The photomicrograph shows the levels of the RONS species. The levels of both NO and ROS species were elevated with the administration of DSS, DMH and DSS+DMH groups. These levels were brought towards normal with the co-administration of celecoxib in these groups (a, b, c, d, e). Means in each segment not sharing a common superscript letter that differed significantly (Duncan’s test): p < 0.01.

We presently found that both the PI3K and angiogenic pathway were upregulated in ulcerative colitis associated colon carcinoma. Also, both of these pathways go hand in hand or co-operate with each other for tumor growth and development and can be corrected with the administration of celecoxib.
Upregulation of MAPK/Erk and PI3K/Akt pathways in ulcerative colitis associated colon cancer

The present chapter incorporated the use of an inflammation inducing agent, dextran sulphate sodium (DSS) and a carcinogenic agent dimethyl hydrazine (DMH) in Balb/c mice, individually as well as in combination, to develop animal model for ulcerative colitis, colon carcinoma as well as colitis-associated colon cancer in 18 weeks. Also, the chemoprevention of these diseases was studied by the administration of a second generation non-steroidal anti-inflammatory drug (NSAID), celecoxib. Numerous epidemiological studies have reported that the long-term use of NSAIDs is associated with a significant decrease in cancer incidence and delayed progression of malignant disease (Fuchs and Ogino, 2013). The expression of Cox-2 and prostaglandins has been associated with various types of cancer and is directly proportional to their aggressiveness including metastasis (Misra and Sharma; 2014). NSAIDs act primarily by the inhibition of the Cox enzyme (Renard et al., 2014). But how these NSAIDs act in case of ulcerative colitis and colitis associated colon cancer is still not clear. We, presently make an attempt to reveal the mechanism underlying colitis associated colon cancer treatment with NSAIDs.

Through a network of proteins, cells respond to the changes in their environmental milieu. This network can be split into many signalling pathways. These pathways carry signals from the environment to the cellular components involved in several pathologies including cancer (Ashton-Beaucage and Therrien; 2010). Extracellular factors such as cytokines and chemokines secreted by inflammatory microenvironment bind to an RTK, leading to the activation of tyrosine kinases, like JAK-3. Upon binding, an RTK dimerizes and autophosphorylates its C-terminal region (Lemmon and Schlessinger, 2010). The resulting phospho-tyrosine residues of JAK-3 serve to activate the MAPK/Erk pathway through a series of kinases including Ras, Raf and MEK (MAPKK) (Udell et al., 2011, Sundaram; 2013). Recent studies have indicated that RTK/ERK pathway might be a key pathway in the development of prostate cancer (Chen et al., 2013). But their role in colitis associated colon cancer is still not known. Thus to identify the role of colitis in colon carcinoma, we studied the protein expression of Jak-3 and p-Erk.
**JAK-3 activated ERK pathway:** Cytokines secreted in the inflammatory microenvironment lead to the activation of tyrosine kinases like JAK-3, which are otherwise activated by NF-κB in the carcinogenic conditions. This JAK-3 initiates the activation of a cascade of kinases including ERK, which further activates the oncogenic PI3K pathway and also generates cell survival and anti-apoptotic signals. JAK-3 also activates its downstream growth factor Stat-3, whose phosphorylated active form enters the nucleus and is a point of convergence for numerous oncogenic signaling pathways. We presently studied the protein expression of JAK-3, p-Stat-3 and ERK by immunofluorescence and western blots and found it to be elevated in the three groups: DSS, DMH and DSS + DMH, although celecoxib co-administration significantly lowered these levels (**Fig. 4.7.1a-d, 4.7.2a-b, 4.7.3a-b**).

**Fig. 4.7.1:**
Fig. 4.7.1: a: The immunofluorescent expression of Jak-3 was found to be aggravated in DSS, DMH and DSS+DMH groups as seen by the green colour of FITC labelled secondary antibody. The counterstained nuclei were red/orange in colour due to PI staining. b: Shows the immunofluorescent staining scores for Jak-3 in the form of stock chart (\(^{a,b,c,d}\) Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test):  \(p < 0.01\)). c: The protein expression of Jak-3, as studied by western blotting was also found to be elevated in the above three groups. These were corrected to a significant extent by the co-administration of celecoxib in these groups. d: Graphical representation of the densitometric analysis of this blot (\(^{a,b,c,d,e}\) Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test):  \(p < 0.01\)).
Fig. 4.7.2: 

Fig. 4.7.2: a: Shows the protein expression of p-Stat-3 in different groups after the fluorescent immunohistochemistry of paraffin embedded tissue sections. These proteins were overexpressed in DSS, DMH and DSS+DMH groups while these were suppressed with the administration of celecoxib in these groups. b: Shows the immunofluorescent staining scores for p-Stat-3 in the form of stock chart (a, b, c, dMeans in each segment not sharing a common superscript letter that differed significantly (Duncan’s test): p < 0.01).
Fig 4.7.3a: The photomicrograph shows the protein expression of p-Erk as studied by immunofluorescent staining. Increased expression was observed in the DSS, DMH and DSS+DMH groups, while it was reverted towards normal with the co-administration of celecoxib in these groups. b: Shows the quantitative analysis of the immunofluorescence of p-Erk (a, b, c). Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
ERK associated PI3K/Akt/ PTEN and Wnt/ β-catenin pathway: The present study explored several components of the PI3K pathway, wherein the immunofluorescent and protein expression of PI3K, p-Akt, PDK1, mTOR, Wnt and β-catenin were found to be elevated. The expressions of PTEN, the negative regulator of this pathway, and GSK3β, a tumor suppressor, were lowered in DSS, DMH and DSS + DMH groups while these levels were brought towards normal with the co-administration of the celecoxib in these groups (Fig.4.7.4-4.7.8).

Fig. 4.7.4:

Fig. 4.7.4: a: Shows the protein expression of various components of PI3K/Akt/PTEN pathway as studied by western blots. An upregulation of PI3K, PDK1, p-Akt, mTOR, WNT and β-catenin and downregulation of GSK-3β were observed in the DSS, DMH and DSS+DMH groups while these effects were brought towards normal by the simultaneous incorporation of celecoxib. b: Graphical representation of the densitometric analysis of these blots (\(^{a, b, c, d, e, f, g}\)) Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): \(p < 0.01\).
We presently studied the protein expression of several components of PI3K/Akt/PTEN pathway in colitis associated colon cancer and found elevated levels of PI3K, Akt, mTOR, PDK1 and β-catenin in the DSS, DMH and DSS+DMH groups. The protein expressions of PTEN as well as GSk-3β were reduced in these groups. Also, Wnt pathway is found to be activated under inflammatory and tumorigenic conditions and further, Wnt expression was upregulated in the above three groups. These effects were decreased by the co-administration of celecoxib in these groups.

Also, Jak-3 is the physiological activator of Signal transducer and activator of transcription 3 (stat 3), a transcription factor with known oncogenic potential (Lin et al., 2005). Activated stat-3 (p-stat 3) has been proposed to be a novel molecular target for therapeutic intervention in malignant neoplasms (Buettner et al., 2002). Recent literature supports the inhibition of Jak-3 signalling leading to the alleviation of inflammation (Kim et al., 2011). Stat 3 is also known to interact with mTOR, a pleiotropic serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription (Moore et al., 1996). We took an insight into this oncogenic Jak-3/stat 3 signalling and found elevated levels of protein expression of jak-3, p-stat 3 and mTOR.

The Ras/Erk and PI3K/Akt/PTEN signaling pathways are the chief mechanisms for controlling cell survival, differentiation, proliferation, metabolism, and motility in response to the extracellular signals from the inflammatory or tumorigenic microenvironment (Mendoza et al., 2011). Both of these pathways are commonly thought to have anti-apoptotic and drug resistant effects on the cells. The Ras/Erk pathway has been reported to be activated in over 50% of acute myelogenous leukemia and acute lymphocytic leukemia, and is also frequently activated in other cancer types (McCubrey et al., 2007). Induced Raf expression can abrogate the cytokine dependence of certain hematopoietic cell lines, a trait associated with tumorigenesis. Also, expression of activated PI3K or Akt has the positive effects on cell survival. Several components of these pathways are mutated or aberrantly expressed in human cancer (e.g., Ras, B-Raf, PI3K, PTEN, Akt) (McCubrey et al., 2006).
Fig 4.7.5a: The photomicrograph shows the protein expression of p-Akt as studied by immunofluorescent staining. Increased expression was observed in the DSS, DMH and DSS+DMH groups, while it was reverted towards normal with the co-administration of celecoxib in these groups. 

Fig 4.7.5b: Shows the quantitative analysis of the immunofluorescence of p-Akt (a, b, c, d). Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
Fig. 4.7.6: a: The immunofluorescent analysis of mTOR shows its elevated expression in DSS, DMH and DSS+DMH groups. These effects were reduced significantly by the co-administration of celecoxib in these groups. b: Shows the quantitative analysis of the immunofluorescence of p-Akt (\(^a, b, c, d\)Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
Fig. 4.7.7a: The photomicrograph shows the upregulation of the expression of β-catenin in the DSS, DMH and DSS+DMH groups while this expression was reduced with the administration of celecoxib in these groups. b: The quantitative analysis of this immunofluorescent expression further supported these microscopic results where maximum score is seen for DMH, DMH+DSS and DSS groups, while it was reverted towards normal with the co-administration of celecoxib (a-h, c, d). Means in each segment not sharing a common superscript letter that differed significantly (Duncan’s test): p < 0.01).
Fig. 4.7.8a: The photomicrograph represents the protein expression of the tumor suppressor PTEN in different groups as studied by immunoflorescence and a decreased expression of PTEN is visualized in the DSS, DMH and DSS+DMH groups while these were brought towards normal with the co-administration of celcoxib in these groups. b: The quantitative analysis of this expression further supports these microscopic results (\textsuperscript{a, b, c}Means in each segment not sharing a common superscript letter that differed significantly (Duncan's test): p < 0.01).
We can, therefore, conclude that both Ras/Erk and PI3K/Akt/PTEN pathways act parallel in case of inflammation mediated tumorigenesis. Also celecoxib, a second generation NSAID, when co-administered with DSS, DMH and their combinatorial group, was found to suppress both Ras/Erk and PI3K/Akt/PTEN pathways.