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Research Article

# STUDY OF BIOMEDICAL EFFICACY OF BETA ASARONE AGAINST PANCREATITIS RATS

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**ABSTRACT**

Albino Wistar rats were divided into IV groups. Group I served as normal and II as negative control which receives normal diet containing 5% fat. Group III and IV rats were treatment groups administered orally with β-asarone 25mg and 50mg/kg body weight/day and also fed with high fat diet containing 25% fat respectively at the last 28 days of the experimental period. Group II-IV were administered with 50µg/kg colchicine interaperitonially to induced acute pancreatitis. Group II rats received 50 µg/kg colchicine. Serum lipase, amylase, cytokine IL-1β, myeloperoxidase activity and antioxidant status in pancreas were assessed. We observed a significant decrease in the activity of serum amylase, lipase, IL-1β B asarone supplementation was found to maintain the activity of myeloperoxidase and antioxidants in pancreas. The optimum protective effect was observed in rats received 50 mg/kg body weight of beta asarone and the histopathological observations added more evidence for our results.

**Keywords:** colchicine, acute pancreatitis, beta asarone

## INTRODUCTION

Pancreatitis is inflammation of the pancreas. It occurs when the enzymes that digest food are activated in the pancreas itself instead of in small intestine. Acute pancreatitis is marked by the sudden onset of right upper quadrant or epigastric pain, whereas chronic pancreatitis is characterized by a recurring or persistent abdominal pain with or without steatorrhea or diabetes mellitus (Harper HJ et al 2011). Chronic pancreatitis is a fibro-inflammatory disease of the pancreas characterized by irreversible morphologic changes that typically cause pain and/or loss of function. Pain occurs in 80% to 90% of patients, and is considered the most important factor affecting quality of life (Braganza et al 2011). Eighty percent of pancreatitis is caused by alcohol and gallstones. Alcohol consumption is a leading cause of both acute and chronic pancreatitis, followed by gallstones and autoimmune diseases etc. (Geokas MC et al 1981).

It is generally believed that pancreatitis is caused by the self digestion of pancreatic acinar cells following the conversion of the inactive trypsinogen to the active trypsin. Many supportive allopathic medicines have been currently used to alleviate the complications associated with inflammation in pancreas (AM Gudgeon et al 1990).The ever increasing mortality due to pancreatitis demands the identification and evaluation of more and more plant based medicines which have fewer side effects. B asarone has been shown to have anti-inflammatory and vasoactive properties (Suvarna shenvi et al 2011). The present study is an attempt to evaluate whether beta asarone could modulate the changes induced in the pancreas of rats administered with colchicine. The dose responsive protective effect of beta asarone on pancreas has also been studied.

## MATERIALS AND METHODS

### Chemicals and reagents

Beta asarone and all other chemicals and solvents used for the analyses were of analytical Grade obtained from sigma Aldrich, Bangalore. Colchicine was obtained from zydus synovia.

### Experimental protocol

Albino rats (Wistar) weighing 175-200g were maintained on 12 hours light and 12 hours dark cycle at 22°C. All animals were individually housed and fed standard rat chow obtained from Hindustan Lever Ltd, Bangalore, India during the acclimation period. Subsequently rats were assigned into 4 groups and fed with rat chow for 28 days. First colchicine was administered for II, III and IV group for a period of 2 days intraperitonially to induced pancreatitis. Then Beta asarone 25 mg/kg/body wt and 50mg/kg/body wt was administered to group III and IV.

Beta asarone was dissolved in normal saline and mixed thoroughly. Beta asarone was administrated orally (Geng Y et al 2010). The work protocol was submitted and approved by The Animal Care Ethical Committee. (XV/VELS/PCOL/03/2000/CPSCEA/IAEC/ 30.10.13). After the experimental period of 28 days, rats were fasted overnight and anesthetized by intramuscular injection of ketamine hydrochloride (30mg/kg body weight) and killed by cervical decapitation. Blood was collected with/without anticoagulant and plasma / serum separated were stored until analysis.

### Tissue homogenate preparation

Immediately after the animal sacrifice, pancreas was removed carefully washed and homogenized in 0.1 M Tris HCl buffer pH-7.4 and centrifuged at low speed to remove any cell debris. The supernatant was used for the determination of glutathione, protein, lipid peroxides and anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).

**BIOCHEMICAL INVESTIGATIONS**

**Determination of serum lipase activity**

Lipase activity in serum was measured by the method of Lowry and Tinsley. The lipolysis reaction was initiated with the addition of serum in 25ml olive oil/triton X - 100 emulsions as substrate. 0.3ml subsamples of reaction mixture were taken at predetermined time intervals and used for the assay of liberated free fatty acids spectrophotometrically at 715 nm. The activity of enzyme was expressed as IU/L.

**Determination of serum amylase activity**

Amylase activity was determined by the method of Gomori[41]. The method was based on the activity of enzyme on substrate starch and the measurement of maltose liberated by using luqol's iodine solution. The colour intensity was measured spectrophotometrically at 640nm and the enzyme activity was expressed as IU/L.

**Determination of myeloperoxidase activity**

Myeloperoxidase (MPO) activity in the pancreatic tissue was measured according to the method of Bradley *et al*. Pre-weighed tissue was homogenized (1:10 w/v) in 0.5% hexadecyltrimethyl ammonium bromide in 50mM potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20 sec. Three freeze / thaw cycles were performed followed by sonication (20 sec in ice bath). The samples were centrifuged at 17000 g (5 min, 4°C) and the enzyme activity was assayed by mixing 0.1 ml of supernatant and 2.9ml of 10mM potassium phosphate buffer (pH 6) containing 0.167g/L o-dianisidine dihydrochoride and 0.0005% hydrogen peroxide. The change in absorbance at 460nm was measured for 4 min using UV visible spectrophotometer. The enzyme activity was expressed as units/mg protein.

**Estimation of glutathione and antioxidant enzymes**

Glutathione (GSH) level was determined by the method of Moron *et al*. Aliquots of plasma or homogenate were mixed with equal volume of ice cold 5% TCA and the precipitated proteins were removed by centrifugation. The supernatant was added to equal volume of 0.2 M phosphate buffer, pH 8.0 and measured at 412 nm. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler. The activity of GPx was expressed as nM of glutathione oxidized / min / mg protein

Superoxide dismutase (SOD) activity was measured according to method the of Kakker *et al.*The inhibition of reduction of nitroblue tetrazolium to blue coloured formazan in the presence of phenazine methosulfate and NADH was measured at 560 nm using n- butanol as blank. The enzyme activity was expressed as units/mg protein. Decomposition of $H_2O_2$ in the presence of catalase (CAT) was kinetically measured at 240nm 30. CAT activity was defined as the amount of enzyme required to decompose 1μM of $H_2O_2$ / min. The enzyme activity was expressed as μM of $H_2O_2$ consumed/ min/ mg protein.

**Western blotting**

The western blot (sometimes called the protein immunoblot) is a widely used analytical technique used to detect specific proteins in a sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are stained with antibodies specific to the target protein. The gel electrophoresis step is included in western blot analysis to resolve the issue of the cross-reactivity of antibodies.

**Histopathology**

For histopathology examination, the pancreatic tissues were excised and rinsed with ice-cold saline solution (0.9% sodium chloride) to remove blood and debris of adhering tissues. The tissues were then fixed in 10% formalin for 24h. The fixative was removed by washing through running tap water and after dehydration through a graded series of alcohols, the tissue were cleaned in methyl benzoate and embedded in paraffin wax. Sections were cut into 5μM thickness and stained with hematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details. Histology was determined for each slide and the average score has been presented in the result section. (Ravikumar et al 2012 and Fathima Cynthia et al 2013)

**Statistical Analyses**

Data were analyzed by using a one way ANOVA method (Dunnett test). Student's t test was performed and results were presented as mean ± S.E.M.

**RESULTS**

**Effect of beta asarone on serum lipase and amylase**

The activities of serum lipase and amylase, the marker enzymes of pancreatic functions are presented in table 1. Serum amylase and lipase activities were increased significantly with β asarone showed decrease in the level of serum amylase and lipase in a dose dependent manner and an optimum decrease was observed in rats received 50mg/kg body weight of asarone.

**Effect of beta asarone on IL-1β and MPO**

Table 2 shows the level of serum IL-1 β and MPO in the pancreas of experimental animals. Rats co-administered with asarone showed a decrease in the level of IL-1 β and a well pronounced decrease was observed in rats received 50mg/Kg body weight of asarone.
The activity of MPO in pancreas was significantly elevated in group III and group IV rats and the level was significantly maintained in rats received 25 and 50mg/Kg body weight of beta asarone.

**Effect of beta asarone on the histology of pancreas**

The histopathological scores in the pancreas of control and experimental rats are shown in Table 3. The pancreas of control rats showed normal architecture without steatosis, inflammation and necrosis. Rats received colchicine showed significant inflammatory and necrotic changes with extensive fibrosis. Beta asarone co administration showed significant reduction in steatosis and inflammation in a dose dependent manner. The protective effect of asarone on pancreas was optimum in rats received 50 mg/kg body weight.

**Effect of Beta asarone on antioxidants in pancreas**

The activities of SOD, CAT and GPx in the pancreas administration are presented in table 4. Treatment received rats showed to maintain the level of antioxidants in a dose dependent manner with the optimum effect at 50mg/kg body weight of beta asarone.

**Table 1. Estimation of serum amylase and serum lipase**

| Samples | Lipase (IU/L) | Amylase(U/L) |
|---|---|---|
| Normal | 121 ± 12.71 | 1490 ± 33.89 |
| Negative control (Colchicine) | 178.2 ± 22.34 | 2308± 84.48 |
| Treatment I (Colchicine+B.A 25 mg/kg) | 124.8 ± 18.2* | 1540 ± 72.40* |
| Treatment II(colchicine+B.A 50mg/kg) | 123.02 ± 12.71** | 1500 ± 20.36** |

**Table 2. Serum Activity levels of serum IL – 1β and myeloperoxidase in the pancreas of experimental animals**

| Groups | IL - 1β (pg/ml) | Myeloperoxidase (Units/mg protein) |
|---|---|---|
| Normal | 11.6 ± 0.7 | 1.86 ±0.06 |
| Negative control (Colchicine) | 23.2 ± 0.9 | 3.42 ±0.12 |
| Treatment I (Colchicine+B.A 25 mg/kg) | 15.0 ± 0.8* | 2.19 ±0.07* |
| Treatment II(colchicine+B.A 50mg/kg) | 13.9 ± 0.7** | 2.14 ±0.11** |

Values are expressed as mean ± SD for six animals in each group. Normal Vs Neg.control, Neg Control Vs Treatment I and II.**p< 0.01*p<0.001, *p<0.01.

**Table 3 Pathological scores in the pancreas**

| Groups | Steatosis | Inflammation | Acinar necrosis | Total score |
|---|---|---|---|---|
| Normal | 0 | 0 | 0 | 0 |
| Negative control (Colchicine) | 2.6±0.27 | 1.0 ±0.16 | 1.3±0.15 | 5.3±0.67 |
| Treatment I (Colchicine+B.A 25 mg/kg) | 1.2±0.17 | 0.6±0.05 | 0.5±0.05 | 2.3±0.31 |
| Treatment II(colchicine+B.A 50mg/kg) | 0.2±0.03 | 0.3±0.023 | 0.14±0.014 | 0.63±0.08 |

**Table 4. Estimation of activity levels of antioxidant enzymes and glutathione from pancreas homogenate**

| Group | Gpx(µg/mg 0f protein) | SOD(unit/min/gm tissue) | Catalase(µmol of $H_2O_2$/min/gm tissue) | GSH(µg/mg of protein) |
|---|---|---|---|---|
| Normal | 1.56±0.19 | 13.68±1.52 | 109.5±15.88 | 13.57±1.49 |
| Negative control (Colchicine) | 0.51±0.05 | 8.24±1.24 | 69±9.23 | 7.23±0.07 |
| Treatment I (Colchicine+B.A 25 mg/kg) | 1.34±0.14* | 11.35±1.37* | 103.42±12.06* | 11.03±1.32* |
| Treatment II(colchicine+B.A 50mg/kg) | 1.39±0.16** | 12.32±1.39** | 104.67±13.39** | 11.21±1.35** |

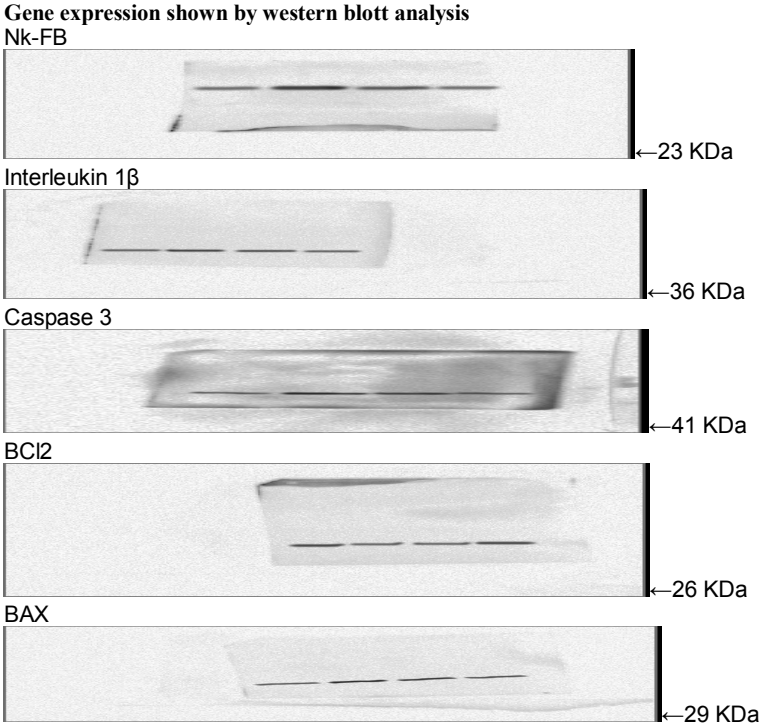
All value are expressed as mean ± SEM (n=6).***P<0.001, **P<0.01,*P<0.05 as compared to Negative control,### P<0.001, ##P<0.01, #P<0.05 as compared to Normal. One-way ANOVA followed by Bonferroni multiple comparison test

**Table 5. Estimation of serum lipid and Triglyceride profile in pancreatic induced rats**

| GROUP | TG(mg/dL) | HDL(mg/dL) | LDL(mg/dL) | VLDL(mg/dL) |
|---|---|---|---|---|
| Normal | 53.16±1.138 | 54.17±0.693 | 17.44±0.40 | 7.64±0.40 |
| Negative control (colchicine 50µg/kg) | 83.5±11.59 | 88.14±0.44 | 36.81±6.75 | 27.78±2.85 |
| Diet+colchicine+B.A25mg/kg | 61.6±5.21* | 62.24±2.30* | 19.09±1.59* | 11.50±1.13* |
| Diet+colchicine+B.A 50mg/kg | 58.92±1.53** | 56.41±3.66** | 18.80±1.31** | 9.45±0.22** |

All value are expressed as mean ± SEM (n=6).***P<0.001, **P<0.01,*P<0.05 as compared to Negative control, ### P<0.001, ##P<0.01, #P<0.05 as compared to Normal. One-way ANOVA followed by Dunnet test

**Gene expression shown by western blott analysis**




## DISCUSSION

### Effect of Beta Asarone in Colchicine induced Pancreatitis on measurement of serum amylase and serum lipase

In present study serum amaylase and serum lipase are generally measured to determine the functionally efficacy of pancreas. These enzymes are synthesized by pancreatic cells which when subjected to injury release the enzyme to blood circulation to elevate levels of serum, (Szalay F 2003).In this study the( colchicine + diet) , negative control ) group showed increase serum level of lipase and amaylase due to cell damage in pancreas (Clavien P et al 1989 ). The role of beta asarone is well proved through its reduction of serum amalyase and lipase activity in dose dependent manner. Beta asarone administration is found to reduce the cell damage as well as the release of enzymes into the blood circulation. The effective dose for beta asarone was found to be 50 mg/kg /bwt. The role of Beta asarone as protective agent is well proved through its effect on reducing the serum lipase and amylase activity in a dose dependent manner (Leser HG et al 1991). ( Colchicine + diet) fed group of animals have shown increased level of lipase and amylase due to cell damage in pancreas (Geetha et al 2013 and Kusano C, et al 2008). Beta asarone administration is found to reduce the cell damage as well as the release of enzymes into blood circulation. Serum amylase and lipase are generally measured to determine the functional efficiency of pancreas (Chen CC et al 1999) .These enzymes are synthesized by pancreatic cells which when subjected to injury release the enzymes to blood circulation and elevating the enzyme level in serum. (Xi Zou et al 2012)

### Effect of Beta Asarone in Colchicine induced Pancreatitis on measurement of serum lipid and serum triglycerides

The dose of beta asarone not only lowered the TG, VLDL, LDL levels but also enhance the cardioprotective lipid HDL in normal and pancreatitis rats in treatment. High level of triglycerides and more importantly LDL cholesterol is the major cause of coronary risk factors ( Jabbar MA et al 1998). Administration of colchicine increased the levels of TG,LDL,VLDL ,HDL where as beta asarone 50mg/kg bd wt lowered TG,LDL,VLDL,HDL levels respectively (Jain et al 2007 and Wang H et al 2009).This is the important finding of the study as pancreatitis is associated with coronary complications (Hotz HG et al 1998 and Lawson EB at 2011).

### Effect of beta asarone on Myelo-peroxidase (MPO) activity of colchicines induced acute pancreatitis

As we have also confirmed histologically in our pancreatitis model, commonly observed changes of pancreatic morphology during pancreatitis include various degrees of acinar cell damage, hemorrhage, and the recruitment of leukocytes into the damaged gland (Bulkley GB, 1983 and lder G et al 1986). MPO is an essential enzyme for normal neutrophil function, and when neutrophils are stimulated by various stimulants, MPO, as well as other tissue damaging substances, is released from the cells. Therefore, MPO is used as an index of tissue neutrophil infiltration (Kettle A J et al 1997). In the present study, the colchicine-induced pancreatitis increase of MPO activity was significantly reduced by beta asarone, suggesting that pancreatic oxidative damage involves the interaction of neutrophils, and the protective effect of beta asarone on the pancreas depends on blockade of neutrophil infiltration (Bomser J 1999). As activation of neutrophils might lead to the generation of reactive oxygen metabolites, the reduction in tissue neutrophil accumulation may also result in reduced lipid peroxidation and attenuated tissue injury. Beta asarone treatment markedly reduced the MPO activity.

### Effect of Beta asarone on anti-oxidant enzymes levels of colchicines induced acute pancreatitis

Antioxidants *in vivo* can be either enzymatic or non enzymatic, counteracts with the formation of ROS and thus protect the cells from the harmful effect of free radicals. Beta asarone was found to reduce the level of glutathione and enzymatic GPx, SOD and CAT. These enzymes are effective in quenching and clearing the toxic free radicals. (Kusano C, et al 2008 and biruk sintayehu B et al 2012).

**Catalase** converts harmful hydrogen peroxide into water and oxygen and protects the tissues from highly reactive hydroxyl radicals (Chance B et al 1992). The reduction in the activity of this enzyme may results in number of deleterious effect due to accumulation of highly toxic metabolites and colchicine administration, which can induce oxidative stress in the cells (Pauli Magnus et al 2005). Beta asarone administration showed significant near to normal value.

**SOD**

SOD which converts superoxide radicals to $H_2O_2$ is widely distributed in cells having oxidative metabolism and is believed to protect such cells against the toxic effects of superoxide anion (Anandan R, et al 1998).Superoxide anions are known to exert destructive effects on cellular components with lipid peroxidation being one such consequence. CAT is a heme protein, which catalyses the direct degradation of hydrogen peroxide to water. It protects the cellular constituents against oxidative damage. The decreased activities of these anti-peroxidative enzymes during colchicine administered in our study are compatible with other studies (Chlebda E et al 2010 and Sreepriya M et al 2001). The decreased activity of these enzymatic antioxidants may be due to the accumulation of $H_2O_2$ which in turn causes the inhibition of these enzymes (Subash S, et al 2008). The reduced activities of these enzymes were normalized upon treatment with beta asarone.

**GSH**

In accordance with the previous reports, which have reported a marked and early depletion of GSH pancreatic tissue in different models of experimental pancreatitis (Dabrowski A et al 1992 and Luthen R et al 1995), we also showed GSH depletion in the pancreatic tissue. GSH plays a role in acinar stimulus-secretion coupling (Stenson WF et al 1983), in the maintenance of the cytoskeleton (Jewell SA et al 1982), and in appropriate protein folding in the endoplasmic reticulum (Scheele G, et al 1982). Thus, depletion of intracellular GSH may contribute to impaired zymogen granule transport and to premature activation of pancreatic proenzymes. Similarly in the present study, following colchicine injection, GSH was depleted; however, beta asarone treatment restoring tissue GSH reduced the severity of pancreatitis. Thus, it is likely that beta asarone increases the total amount of intracellular GSH and has an important role in the maintenance of this crucial antioxidant (Akyuz et al 2009).

**Gpx**

GPx catalyses the reduction of hydrogen peroxide and hydroperoxide to non-toxic products and scavenges the highly reactive lipid peroxides in the aqueous phase of cell membrane. GPx and the cellular NADPH-generating mechanisms together form a system for removing hydroperoxides from the cell (Subash S,et al 2008). The decreased activity of GPx in toxicated group might be correlated to the decreased availability of its substrate GSH. After oral treatment with beta asarone, the GPx levels significantly improved to near normal. Glutathione a prime antioxidant that can scavenge free radicals and prevent the formation of hydrogen peroxide was found to be maintained significantly by beta asarone supplementation. The decreased concentration of pancreatic GSH was observed in our study in colchicine + diet fed rats might be due to its elevated consumption or reduced formation. Many pancreatic disorders are associated with depleted GSH level in various organs. Enzymes GPx, CAT and SOD were active in clearing the free radicals and their depletion are also observed in many pathological conditions affecting pancreas. Our results are in accordance with the other reports which showed that beta asarone scavenge free radicals and also play an important role in maintaining endogenous antioxidant status. Absorption of nutrients and thereby reduce energy formation. Reduced food consumption might also be due to the functional disturbance in gastrointestinal tract. Beta asarone supplementation was found to improve food consumption as well as to maintain net weight gain showing its modulatory effect on group II induced changes in rats.

**Beta asarone effect on gene expression by western blotting of colchicine induced acute pancreatitis**

Apoptosis is a teleologically beneficial form of cell death in acute pancreatitis. However, the exact mechanism by which the induction of apoptosis protecting against progression toward acute pancreatitis is still not clear. This study shows that beta asarone induces phagocytosis of apoptotic acinar cells by positive macrophages during acute pancreatitis. The anti-inflammatory effect of the phagocytosis is mediated by the production of pancreatic anti inflammatory mediators such as IL-β Besides the anti-inflammatory response of phagocytosis, suppressing levels of pancreatic pro-inflammatory mediators also contributes to asarone mediated-protection against rat acute pancreatitis. However, the large complexity of pattern of recognition receptors depends on the means of induction of cell death, cell type including both apoptotic cells and phagocytes, as well as the surrounding microenvironment (Savill, Dransfield *et al.* 2002; de Almeida and Linden 2005).

**IL-1**

Interleukin (IL) IL-1 is a pro-inflammatory cytokine generated by the pancreas that plays an important role in the early stage of severe acute pancreatitis. In a animal model, the IL-1 receptor antagonist (IL-1) has been found to decrease case fatality by 30% in addition, the IL-1 receptor can markedly lower the concentrations of IL-6 and TNF-α .(Fink et al) administered the IL-1 receptor antagonist before inducing the pancreatitis model and found that the IL-1 receptor block markedly lowered the release of amylopsin and pancreatic necrosis in a dose-dependent manner.

The generation of IL-1 β formed from IL-1 through the mediation of IL-1 convertase (ICE). IL-1β and TNF-a have many of the same biological activities, including pyrogen functions, the promotion of cell catabolism, the production of protein in the acute reaction period, effecting the secretion of PGI2 by epithelial cells and platelet activating factor, among others, that will cause the expansion of the inflammation area and increase the levels of inflammatory mediators, destructive enzymes and ROS secretion. IL-1beta can interact with TNF-α to induce or aggravate organ injury.

IL- 1β binds to the receptors IL- 1 R1 / IL- 1R1 ACP heterodimer which then initiates the signaling cascade resulting in the translocation of the transcription factor nuclear factor kappa β into the nucleus, where it induces the transcription of pro and anti inflammatory gene including inducible nitric oxide synthatase, IL-1R and COX 2 catalyses the conversion of arachidonic acid (AA) to prostaglandin H2. (Leusheshi ,et al1998 and Vincenti, et al 2001).
We could find in our study significant increase in the serum level of IL- 1β colchicine fed rats when compared to normal rats. Beta asarone supplementation is found to reduce the level of interleukins significantly in group III and IV rats. A significant reduction in the serum concentration of IL- 1β shows potent anti- inflammatory nature of beta asarone interleukin-1 converting enzyme.( Geetha et al 2013 )

**NF-kB.** NF-kB is a critical transcription factor for inflammatory mediator induction (Maqbool et al., 2013; Olajide et al., 2013). The specific blockage of NF-kB activity or knockout of its gene in microglia suppresses production of the inflammatory cascade against LPS stimulation (Jayasooriya et al., 2011). Activating NF-kB is necessary to induce the iNOS and COX-2 genes. Activating

NF-kB with LPS stimulates the expression of proinflammatory mediators (Alderton et al., 2001). NF-kB is retained in the cytoplasmof un-stimulated cells by binding to IkB- α. Activation of NF-kB occurs via phosphorylation of its endogenous inhibitor NFkB- α resulting in the release and nuclear translocation of active NF-kB. In present study, beta asarone (Hyung-Woo Lim et al 2014) stimulation leads to translocation of the NF-kB subunit p65 to the nucleus in BV-2 microglial cells. However, pretreatment with b-asarone suppressed NF-kB activation and IkB-α degradation (Liu and Malik 2006) .Our results demonstrate that b-asarone, suppressed microglial activation by inhibiting pro-inflammatory cytokines via NF-kB signaling in vitro. (Rakonczay *et al.* 2008).

**Caspase -3**

Caspase 3 belongs to large family of cellular cystine proteases, known collectively as caspases for their preferential ability to cleave cellular substrates after aspartate residues. The cleavage of caspase specific substrates result in biochemical destruction of the cell and phenotypic changes associated with apoptosois (Yao et al 2007). A decrease in mitochondrial transmembrane potential, release of cytochrome *c* into the cytosol, and cleavage of procaspase-3 to its active form preceded the activation of caspase-3 and, moreover, all of these events began earlier and/or proceeded faster in cells treated with colchicine (AM Gorman et al 1999).Our results suggest beta-asarone administration attenuated colchicine induced expression of activated caspase-3, suggesting that it exerts protective effects against colchicine induced neuronal apoptosis by affecting the execution phase of apoptosis.( Y Geng et al 2010)

**BCL 2**

Besides caspases, we focused on Bcl-2 family because they serve as vital regulators of the mitochondrial pathway involved in apoptosis (Reed, 1998). The Bcl-2 protein, known as an anti-apoptotic protein, binds to the outer membrane of the mitochondria and prevent the release of cytochrome c. (Y Geng et al 2010). Our results suggest that the decreased ratio of Bcl-2 to Bax might be a key indicator in β-asarone inducing apoptosis of cells. (Xi Zou et al 2012)

**BAX**

Bax protein is identified to be pro-apoptotic effectors and responsible for permeabilizing the membrane due to damaging cellular stress (Ewings et al., 2007). Our results demonstrated that Bax protein levels increased gradually in a dose-dependent manner within total cell lysates. A significant decrease of Bax ratio was observed when cells were treated with β-asarone as compared to the colchicine group.( J liu et al 2010). Therefore, β-asarone mediated a cascaded series of molecular events that led to an attenuated level of Bcl-2, augmented level of the pro-apoptotic protein Bax, and activation of the executer apoptosis enzyme caspase-3. (Xi Zou et al 2012).

**CONCLUSION**

βeta asarone modulates the abnormal changes induced by simultaneous administration of colchicine and diet by minimizing amylase and lipase release from pancreas into circulation, myeloperoxidase activity, cytokine production by maintaining the antioxidant status in the glandular organ. Among the two doses of beta asarone studied, 50 mg/kg body weight was found to be most effective in modulating the changes during colchicine induced pancreatic injury. The serum lipid and triglycerides activity was found to be significant at the dose of 50mg/kg beta asarone in colchicine induced pancreatitis rats. Analysis of genes such as (Bax, Bcl2, catalase 3,NKF-β and IL1 β) through western blotting led us to prove that beta asarone has its protective effects.

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