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Research Article

THE MODULATING EFFECT OF METHANOLIC SEED EXTRACT OF *NIGELLA SATIVA* ON ETHANOL AND HIGH FAT DIET INDUCED PANCREATIC INJURY IN RATS

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ABSTRACT

Objective: In this study, the dose dependent effect of methanolic seed extract of *Nigella sativa* (MENS) on ethanol (EtOH) and high fat diet (HFD) induced pancreatic inflammation in rats was investigated.

Methods: Male albino Wistar rats were separated into 7 groups. Group 1and 2 rats were fed normal diet (5% fat) and group 3-7 rats were fed HFD (25% fat) and also administered EtOH (8-12 kg/g/day) for 90 days. In addition, group 4-7 rats were received 50, 100, 200 and 300 mg/kg b. w of MENS and group 2 rats were administered MENS (300 mg/kg b.w) from 31st day.

Results: We determined the activities of serum lipase and amylase and oxidative stress markers, inflammatory markers and caspase-1. MENS co administration significantly decreased serum lipase and amylase activities, oxidative stress markers and inflammatory markers in EtOH and HFD fed rats in a dose dependent manner. Rats co administered with MENS have shown decreased level of caspase-1 activity.

Conclusions: MENS at the dose of 200 mg/kg b.w has a pronounced protective effect on EtOH and HFD induced pancreatic injury in rats. This study revealed that the optimum dose of 200 mg/ kg b.w of MENS can be used for further study to evaluate the mode of pancreato protective action in rat model.

Keywords*:* Caspase-1, High fat diet, *Nigella sativa*, Pancreatitis, Proinflammatory cytokines.

INTRODUCTION

Chronic pancreatitis (CP) is an inflammatory disease characterized by severe abdominal pain and progressive destruction of the pancreatic tissue leading to both exocrine and endocrine insufficiency [1]. The pathogenesis of pancreatitis is associated with activation of digestive enzymes, generation of free radicals and release of proinflammatory cytokines [2]. The most common etiological factors of pancreatitis are alcohol abuse, gall stone migration and genetic mutations [3]. Chronic alcoholism is the most important cause of CP and 90% cases of CP are caused by long-term alcohol consumption. Ethanol (EtOH) is oxidized within the acinar cells and the metabolites formed alter the intracellular redox state and generate reactive oxygen species (ROS). Oxidative stress caused by ROS has shown to play pivotal role in the pathogenesis of alcohol induced pancreatic injury [4]. The progression of inflammation and necrosis in pancreas is mediated by proinflammatory cytokines such as IL-1β, IL-18 and TNF-α [5]. Caspase-1 is a cysteine protease involved in the maturation of IL-1β and IL-18 and inhibition of caspase-1 activation has been shown to reduce the pancreatic inflammation [6].

Even though alcohol consumption is the major cause of pancreatitis, only 5% of alcohol abusers develop alcoholic pancreatitis because it requires additional factors for the progression of pancreatitis [7]. Fat rich diet is one such factor that increases the severity of pancreatitis. Currently there is no specific treatment to overcome the complications caused by oxidative stress and inflammation in pancreatitis except the supportive therapy.

Herbal medicines have been focused as a source of curative remedy based on religious and cultural traditions. Millions of people in the Mediterranean region and the Indian subcontinent use *Nigella sativa* seeds as a protective and curative remedy for many ailments including gastrointestinal tract disorders. *N. sativa* seed extract have been used to treat abdominal pain, cough, diarrhea, rheumatism and related inflammatory disorders [8]. Recent studies showed that the pharmacological activities of *N. sativa* seeds are due to the presence of thymoquinone. In addition the seeds also contain alkaloids such as nigllidine, nigellimine, nigellicine, terpenoids, saponins and crude fibers [9].

The aim of the present study is to investigate the effective dose of methanolic seed extract of *N.sativa* (MENS) on EtOH and high fat diet (HFD) induced pancreatic injury in rats.

MATERIALS AND METHODS

Chemicals and reagents

ELISA kits for IL-1β and caspase-1 (Abcam) and for IL-18 (Invitrogen) were used. All other chemicals and solvents used for the analysis were of analytical grade.

Preparation of MENS

N. sativa seeds were purchased from local market and its authenticity was verified by Dr. P. Jayaraman, Director, Plant anatomy research centre, Chennai (PARC/2011/994). The dust free seeds were coarsely grounded into homogenous powder. The MENS was prepared by soaking 150 g of homogenous powder in 150 ml of methanol and kept at room temperature for 7 days. The mixture was filtered using Whatman filter paper no.1 and evaporated till dryness [10].

Animals and diet composition

Male albino Wistar rats weighing 175-200 g were (12:12 h L:D cycle at 22 °C and 50% relative humidity) individually housed and fed standard rat chow obtained from M/S: Provimi Animal Nutrition India Pvt Ltd., Bangalore, India, containing 5% fat. HFD (25% fat) was prepared in our laboratory with required amount of mineral and vitamin mix. Soybean oil was substituted for the supply of essential fatty acids. The energy given by the normal diet is 3.43 Kcal/g and by the HFD along with EtOH is 5.24 Kcal/g. The carbohydrate content in HFD was adjusted isocalorically based on the calories given by EtOH (8 or 12 g/kg/day).

Experimental protocol

Subsequently rats were separated into 7 groups. Group 1 and 2 rats were fed standard rat chow for 90 days. Group 3-7 rats were fed HFD containing 25% fat and administered aqueous EtOH equivalent to 8 g/kg/day for the first 4 weeks and 12 g/kg/day for the remaining days according to the report of Tsukamoto *et al* [11]. Group 4–7 rats were received 50, 100, 200 and 300 mg/kg body weight respectively of MENS for the last 60 days of the experimental

period. Group 2 rats were administrated with 300 mg/kg body weight of MENS for the last 60 days. The work protocol was approved by The Animal Care Ethical Committee.

Sample collection

After the experimental period, rats were fasted overnight and anesthetized by intramuscular injection of ketamine hydrochloride (30 mg/kg body weight) and killed by cervical decapitation. Blood was collected and plasma/serum separated was stored until analyses. The remaining portion of pancreas was 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 (GSH), caspase-1, lipid peroxides and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Biochemical investigations

Determination of serum lipase activity

Serum lipase (EC: 3.1.1.1) activity was determined by the method of Lowry and Tinsley [12]. The addition of serum in 25 ml olive oil/triton X 100 emulsion initiated the lipolysis reaction. Subsamples 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

The activity of amylase (EC: 3.2.1.1) was measured according to the method of Gomori [13]. The activity is measured by determining the amount of starch it can split by using lugol's iodine solution. The color intensity was measured spectrophotometrically at 640 nm and the enzyme activity was expressed as IU/L.

Determination of oxidative stress markers

The level of lipid peroxides in plasma and pancreas was determined by measuring thiobarbituric acid-reacting substances (TBARS) [14]. The value was expressed as nM/mL plasma and nM/100 mg tissue protein. The peroxide content was measured in plasma by using FOX 2 method [15] with minor modifications. The FOX 2 test system is based on oxidation of ferrous ion to ferric ion by various types of peroxides present in the samples, to produce a coloured ferric-xylenol orange complex whose absorbance was measured at 560 nm. Total antioxidant capacity (TAC) was determined by the method of Miller *et al* [16]. Decolourization of assay mixture containing 2, 2'-azino bis 3-ethyl benzo-thiazoline-6-sulfonate and the sample was monitored by measuring the absorbance at 734 nm. The ratio of total peroxides to TAC was calculated as oxidative stress index (OSI).

Estimation of GSH and antioxidant enzymes

GSH was determined by the method of Moron *et al* [17]. Aliquots of plasma or homogenate were mixed with equal volume of ice cold 5% trichloro acetic acid and the precipitated proteins were removed by centrifugation. The supernatant was used for the assay by treating with nitroblue tetrazolium in 0.2 M phosphate buffer, pH 8 and measuring at 412 nm. GPx (EC: 1.11.1.9) was assayed by the method of Flohe and Gunzler [18]. The activity of GPx was expressed as nM of GSH oxidized/min/mg protein.

SOD (EC: 1.15.1.1) activity was measured according to method of Kakker *et al* [19]. The inhibition of reduction of nitroblue tetrazolium to blue coloured formazan in 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 hydrogen peroxide (H_2O_2) in the presence of CAT (EC: 1.11.1.6) was kinetically measured at 240 nm [20]. The enzyme activity was expressed as μM of H_2O_2 consumed/min/mg protein.

Inflammatory markers

Assay of myeloperoxidase (MPO) activity

MPO (EC: 1.11.1.7) activity in the pancreas was measured according to the method of Bradley *et al* [21]. Pre-weighed tissue was homogenized (1:10 w/v) in 0.5% hexadecyltrimethyl ammonium bromide in 50 mM potassium phosphate buffer (pH 6) before sonication in an ice bath for 20 sec. Three freeze / thaw cycles were performed followed by sonication (20 sec in icebath). 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 2.9 mL of 10 mM potassium phosphate buffer (pH 6) containing 0.167 g/L o-dianisidine dihydrochloride and 0.0005% H_2O_2. The change in absorbance at 460 nm was measured for 4 min using fluoro spectrophotometer. The enzyme activity was expressed as units/mg protein.

Assay of IL-1β

The assay of IL-1β was performed according to manufacturer's instructions (ab100767). Aliquots of standard or serum were pipetted into IL-1β antibody precoated wells and IL-1β present in the sample form complex with the immobilized antibody. The wells were washed and biotinylated secondary antibody was added. The unbound biotinylated antibody was washed and added HRP-conjugated streptavidin to the wells. The wells were washed again and TMB substrate solution was added. The intensity of the colour developed was proportional to the amount of IL-1β present in the sample. The stop solution changes the colour from blue to yellow, and the intensity of the colour was measured at 450 nm. The activity of IL-1β was expressed as pg/mL.

Assay of IL-18

The assay was carried out as per the instruction of kit manual (KRC2341). The serum sample and standards were pipetted into antibody immobilized wells. After the incubation, biotinylated secondary antibody was added. After removal of excess secondary antibody, streptavidin-peroxidase was added. Then the substrate solution was added to react with the bound enzyme to produce colour. The intensity of this colour was measured spectrophotometrically at 450 nm. The activity of IL-18 was expressed as pg/mL.

Assay of caspase-1

Caspase-1(EC: 3.4.22.36) activity was determined in serum or pancreatic extract, prepared according to the method of Thornberry *et al* [22] as the enzyme source. Briefly, the pancreas was homogenized in a lysis buffer (25 mM HEPES [pH 7.5], 1 mM EDTA, 10 μg of aprotinin/mL, 10 μg of leupeptin/mL, 2 mM dithiothreitol) at 5 mL/100 mg of pancreas tissue. The extract was centrifuged at 15,000 g for 30 min at 4 °C and the supernatant was centrifuged again at 200,000 g for 1 h at 4 °C. The cytosol was used for the assay of caspase-1. The assay is based on the spectrophotometric detection of the chromophore p-nitroanilide (p-NA) formed from the labeled substrate YVAD-p-NA. The p-NA light emission can be quantified spectrophotometrically at 400 nm.

Statistical analyses

Data were analyzed by using commercially available statistics software package (SPSS for window V.10). The statistical significance of mean values between different groups was determined by applying one way ANOVA with *post hoc* Bonferroni test and the p value < 0.05 was considered as significant.

RESULTS

Effect of MENS on pancreatic marker enzymes

The activities of pancreatic marker enzymes such as lipase and amylase of experimental animals are depicted in table 1. The enzyme activities were found to be elevated (p=0.000) in EtOH and HFD fed rats than in control rats. MENS co administration significantly (p=0.000) decreased the enzyme activities in a dose dependent manner. The optimum effect was observed at the dose of 200 mg/ kg body weight.

Effect of MENS on oxidative stress markers

The level of TBARS, total peroxide, TAC and OSI are given in table 2. MENS co administration significantly decreased the level of TBARS, total peroxide and OSI in EtOH and HFD fed rats. TAC was found to be low in EtOH and HFD fed rats when compared to rats which were co administered with MENS at different concentration and optimum effect was observed with 200 mg/kg body weight of MENS.

Table 1: Activity of serum amylase and lipase in experimental rats

Groups	Lipase (IU/L)	Amylase (IU/L)
Control	125 ± 14.8	1500 ± 184
MENS control	123 ± 17.8 NS	1350 ± 220 NS
EtOH&HFD	190 ± 18.6*	2700 ± 271*
EtOH&HFD+ MENS (50 mg/kg b.w.)	173.10±20.2@	2100±210#
EtOH&HFD+ MENS (100 mg/kg b.w.)	150.11±20.7*	1800±219*
EtOH&HFD+ MENS (200 mg/kg b.w.)	127.53 ± 18.3*	1600 ± 207*
EtOH&HFD+ MENS (300 mg/kg b.w.)	126.10±15.4*	1550±162.4*

Values are expressed as mean ± SD for 6 rats in each group. For comparison group 1 vs group 3, group 1 vs group 2, group 3 vs group 4-7 are considered. *p=0.000, #p=0.04, $p=0.774,

@p=0.006, NS = Non significant.

Table 2: Effect of various concentrations of MENS on the level of TBARS, peroxide content, TAC and OSI in rats

Groups	TBARS Plasma (nM/mL)	Peroxide (mM/L)	TAC (mM trolex eq./L)	OSI (peroxide/ TAC)
Control	0.163 ± 0.01	180.1 ± 20.53	346.66 ± 47.83	0.54±0.05
MENS control	0.17 ± 0.02NS	192.64 ± 28.70 NS	331.02 ± 42.03 *	0.58 ±0.07 NS
EtOH&HFD	0.44 ± 0.04*	298.03 ± 44.70*	224.33 ± 26.42*	1.32 ±0.19*
EtOH&HFD+ MENS (50 mg/kg b.w.)	0.40±0.05 NS	260.14±26.53@	278±41.70*	1.07±0.13 *
EtOH&HFD+ MENS (100 mg/kg b.w.)	0.32±0.03 *	244.52±33.49*	301.72±42.82*	0.72±0.09*
EtOH&HFD+ MENS (200 mg/kg b.w.)	0.19 ± 0.02*	198.62 ± 25.42*	328 ± 33.14*	0.59 ±0.08*
EtOH&HFD+ MENS (300 mg/kg b.w.)	0.18±0.02*	194.04±35.80*	330±40.59*	0.58±0.08*

Values are expressed as mean ± SD for 6 rats in each group. For comparison group 1 vs group 2, group 1 vs group 3, group 3 vs group 4-7 are considered. *p=0.000, @p=0.026, NS = Non significant

Effect of MENS on antioxidants level

The activities of SOD, CAT, GPx and GSH in pancreas of experimental animals are presented in table 3. In EtOH and HFD fed rats the level of GSH was decreased significantly (p=0.000) than in MENS co administered rats. MENS co administration in EtOH and HFD fed rats was found to maintain the level of antioxidants in a dose dependent manner with the optimum effect at 200 mg/ kg body weight of MENS.

Table 3: Activity of antioxidant enzymes and GSH in rats administrated with various concentrations of MENS

Groups	GPx (nM of GSH oxidized/min/mg protein)	SOD (units/mg protein)	CAT (μM H_2O_2 consumed/min/ mg protein)	GSH (mg/g protein)
Control	1.67 ± 0.2	15 ± 1.3	115±15.18	15±2.04
MENS control	1.52 ± 0.2*	16 ± 1.8 NS	118.5±10.97NS	16±1.59 NS
EtOH&HFD	0.40 ± 0.05*	8 ±1.1*	63±7.61*	5.7±1.05*
EtOH&HFD+ MENS (50 mg/kg b.w.)	0.57 ± 0.09*	10 ± 1.33 $	80±12.37*	8±0.89 NS
EtOH&HFD+ MENS (100 mg/kg b.w.)	0.89±0.09*	12 ± 1.35*	98±13.53*	10.8±1.43a
EtOH&HFD+ MENS (200 mg/kg b.w.)	1.48 ± 0.13*	14.01 ± 1.80*	113±12.95*	14.1±1.23*
EtOH&HFD+ MENS (300 mg/kg b.w.)	1.51 ± 0.14*	14.35 ± 1.82 *	113.9±14.65*	14.8±1.67*

Values are expressed as mean ± SD for 6 rats in each group. For comparison group 1 vs group 2, group 1 vs group 3, group 3 vs group 4-7 are considered. *p=0.000, $p=0.029, @p=0.025, #p=0.018, ap=0.003, NS =Non significant.

Effect of MENS on inflammatory markers

MPO activity in the pancreas of experimental animals was depicted in Figure 1. The activity of MPO was elevated in EtOH and HFD rats when compared to MENS co administered rats. The effect was found to be concentration dependent.

Serum and pancreatic caspase-1 activities of various groups were shown in Figures 2 (a) and (b) respectively. In EtOH and HFD rats, the activity of serum and pancreatic caspase-1 were found to be increased significantly (p=0.000) when compared to control rats. MENS co administration significantly reduced the activity of caspase-1 in EtOH and HFD fed rats. The dose of 200 mg/kg body weight of MENS showed significant decrease in caspase-1 activity.

Serum IL-1β and IL-18 levels in experimental animals are presented in Figures 3(a) and 3(b). MENS co administered rats showed decreased level of these proinflammatory cytokines when compared to EtOH and HFD fed rats. More pronounced effect was observed at 200 mg/kg body weight of MENS when compared to other doses.

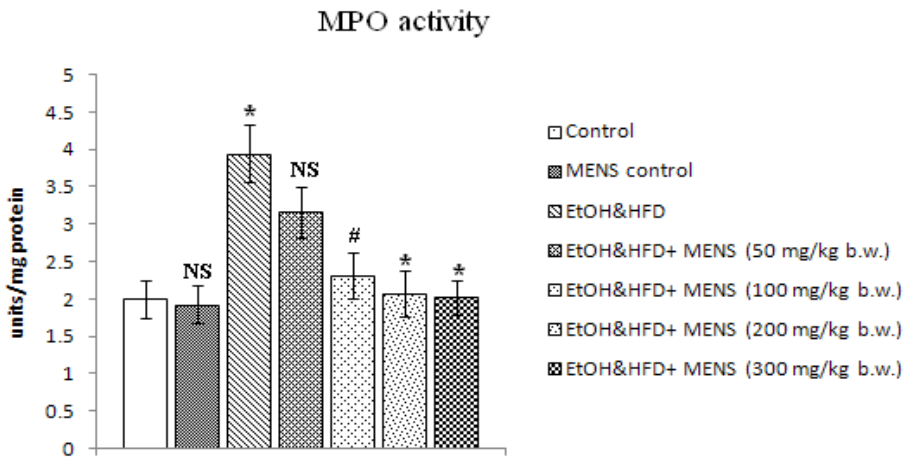

Fig. 1: Changes in the activity of pancreas MPO in EtOH and HFD received rats with/without MENS co administration.

Values are expressed as mean ± SD for 6 rats in each group. For comparison group 1 vs group 2, group 1 vs group 3, group 3 vs group 4-7 are considered. *p=0.000, NS= Non significant.

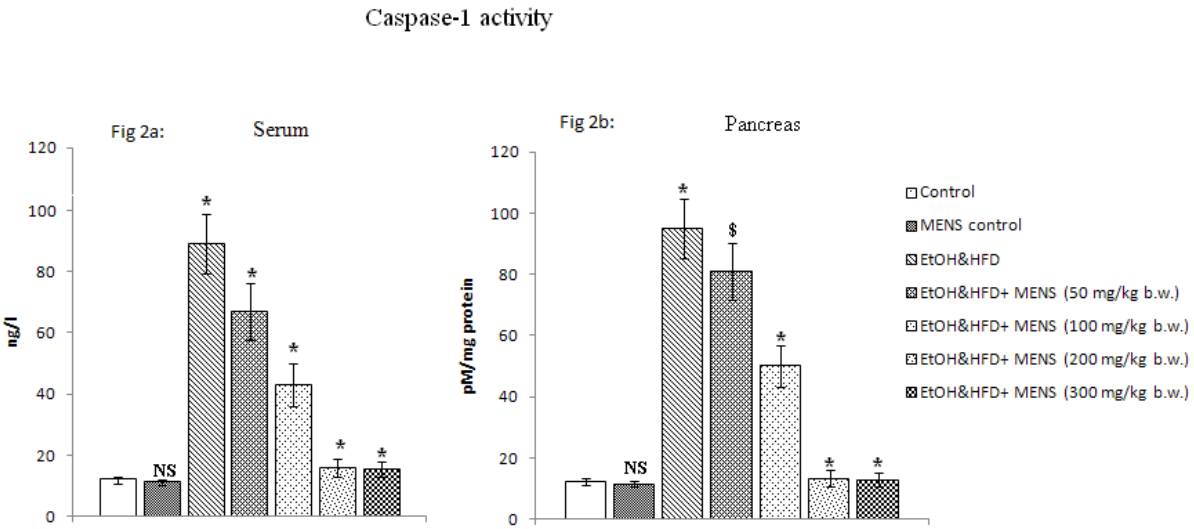

Fig. 2a and 2b: Changes in the activity of serum and pancreatic caspase-1 in EtOH and HFD received rats with/without MENS administration.

Values are expressed as mean ± SD for 6 rats in each group. For comparison group 1 vs group 2, group 1 vs group 3, group 3 vs group 4-7 are considered. *p=0.000, NS – Non significant.

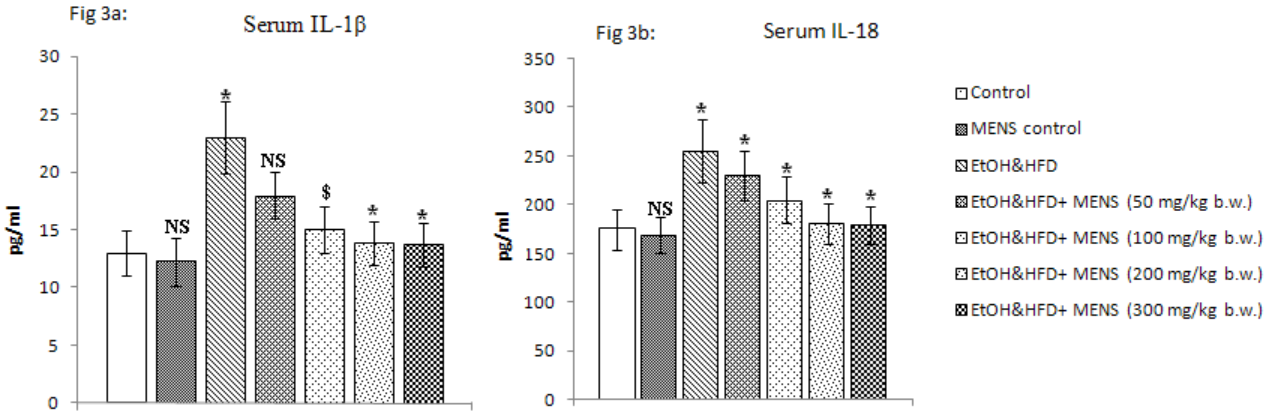

Fig. 3a and 3b: Changes in the activity of serum IL-1β and IL-18 in EtOH and HFD received rats with/without MENS co administration.

Values are expressed as mean ± SD for 6 rats in each group. For comparison group 1 vs group 2, group 1 vs group 3, group 3 vs group 4-7 are considered.*p=0.000, $p=0.029, NS=Non significant.

DISCUSSION

CP is one of the most serious complications of alcohol abuse. About 70% of CP is due to alcohol abuse [23]. Alcohol needs a cofactor to increase the severity of pancreatitis. HFD is one such factor which predisposes the pancreatic acinar cells for inflammation and injury [24]. Hence in the present study, we used EtOH along with HFD to induce pancreatitis in rats.

Alcohol exerts a number of toxic effects on acinar cells through by its metabolites. Acinar cell oxidizes EtOH via both oxidative and non-oxidative pathway. In oxidative pathway, EtOH is metabolized predominately by alcohol dehydrogenase and generate acetaldehyde and ROS. Through the non-oxidative pathway of EtOH metabolism, fatty acid ethyl esters accumulate in pancreas. Acetaldehyde, fatty acid ethyl esters and ROS together have been shown to cause deleterious effects on pancreas. Acetaldehyde induces morphological changes in pancreas and it also interferes with the binding of cholecystokinin to its cellular communication sites. Fatty acid ethyl esters increase the fragility of lysosomes which release the hydrolyzing enzymes to damage sub cellular organelles [25].

The acinar cell produces digestive enzymes as inactive precursors, packed into zymogen granules and segregated from cellular components. Several studies have shown that alcohol consumption increases the secretion of digestive and lysosomal enzymes and concurrently the fragility of lysosomes and zymogens. ROS destabilize zymogen granules and lysosomes and potentially increasing the intracellular activation of digestive enzymes [26]. The premature activation of digestive enzymes within pancreatic acinar cells is the critical event in pancreatitis [27]. We found increased level of serum lipase and amylase in EtOH and HFD fed rats. MENS co administration significantly reduced the serum level of these enzymes showing protective action on pancreas.

Persistent oxidative stress may play a role in the development and progression of CP. Lipid peroxidation represents an important manifestation of oxidative stress. Lipid peroxidation is initiated when free radicals interact with polyunsaturated fatty acids in cell membrane and form hydroperoxides. This may cause disturbances in cellular fluidity and membrane integrity that leads to disintegration of cell and necrotic cell death. Schoenberg *et al* reported high level of MDA in pancreas of rats subjected to experimental pancreatitis [28]. Peroxidation of membrane lipids might lead to the disturbances in the transport of digestive enzymes along with tissue injury [29]. In EtOH and HFD fed rats, the level of TBARS and hence OSI was significantly increased when compared to those of MENS co administered rats. TAC level was found to be low in EtOH and HFD fed rats than in MENS co administered rats at different concentrations. Among the doses studied, 200 mg/kg body weight of MENS was found to reduce the production of ROS significantly.

Antioxidants effectively remove ROS *in vivo* and natural antioxidant enzymes including SOD, CAT and GPx protect the cells from oxidative damage. SOD is the first line defense antioxidant enzyme against the deleterious effects of oxygen radicals and it scavenges ROS by catalyzing the dismutation of superoxides to H_2O_2 [30]. CAT plays an important role against the harmful effects of lipid peroxidation. These enzyme activities were decreased in EtOH and HFD fed rats when compared to control rats. The decrease in SOD and CAT activity leads to accumulation of highly reactive free radicals that causes instability in membrane structure and function. MENS co administration significantly reduced the formation of free radicals in the pancreas of EtOH and HFD fed rats.

GSH is an important non-protein thiol that scavenges H_2O_2 and other peroxides. GSH together with glutathione dependent enzyme GPx, efficiently scavenges toxic free radicals and convert them into non toxic compounds [31]. Oxygen intermediates generated during the metabolism of EtOH leads to consumption of GSH and this depletion results in increased susceptibility of cells to oxidative stress. The consumption of intracellular GSH also may lead to premature activation of pancreatic pro enzymes [32]. In the present study, MENS (200 mg/kg body weight) co administration significantly restored GSH and maintained the other antioxidants level significantly.

MPO is a hemeprotein that is stored in azurophilic granules of polymorphonuclear neutrophils and macrophages. MPO catalyzes the conversion of chloride and H_2O_2 to hypochlorite and actively in neutrophils during inflammatory condition. It is a well known marker of inflammation. MPO is an essential enzyme for neutrophil function and it is released from the cells when neutrophils are stimulated. MPO is also used as a biomarker of tissue neutrophil infiltration. During inflammation neutrophils activation might lead to the generation of ROS and lipid peroxidation [33]. MENS co administration reduced the MPO activity in EtOH and HFD fed rats showing that MENS contains bioactive compounds which are anti-inflammatory in nature.

Several studies suggested that cytokines are the central mediators in local and systemic complications of pancreatitis [34]. Based on clinical observations, proinflammatory cytokines are shown to be elevated in pancreas to enhance the local tissue destruction [35]. Caspase-1 is a cysteine protease involved in the processing of pro IL-1β and pro IL-18 during inflammation. Caspase-1 is synthesized as an inactive zymogen and activated by the molecular assembly of protein complex known as inflammasomes. These inflammasomes are activated by ROS, uric acid and ATP in response to tissue injury [36]. Caspase-1 is a tetramer consists of a larger (p20) and smaller (p10) subunits. Inhibition of caspase-1 activation has been shown to reduce the severity of pancreatitis [6]. The activity level of caspase-1 was significantly decreased in MENS co administered rats. This shows that MENS has the ability to inhibit the activation of caspase-1 in EtOH and HFD fed rats.

The major function of caspase-1 is the conversion of pro forms of IL-1β and IL-18 into their active forms. Caspase-1 dependent activation of IL-1β is known to influence neutrophil activation that causes cell injury [37]. IL-1β, a pro inflammatory cytokine is an important mediator of the inflammation in pancreatitis. It is produced by activated lymphocytes and monocytes and initiates the inflammatory cascade [38]. In this study, EtOH and HFD fed rats showed increased level of serum IL-1β and 200 mg/kg body weight of MENS co administration significantly reduced IL-1β level in serum when compared with that of rats received other doses.

IL-18 is called as interferon-γ inducing factor, shares striking similarities with IL-1β concerning structure, functions which induce the synthesis of each other in inflammation [39]. The level of IL-18 was elevated in CP which may be accounted for the development of complications such as multiple organ failure and septic shock at the final stage of the disease. IL-18 level has been shown increased in pancreatitis and its concentrations closely correlated with the development of pancreatitis [40]. MENS co administration reduced the level of serum IL-18 in EtOH and HFD fed rats.

N. sativa seed extract has been used traditionally for rheumatism, liver diseases and various inflammatory diseases. The effect of this black seed on human health has been investigated by many researches in recent days. This study provides the scientific evidence for the pancreato protective action of *N. sativa* seed extract in EtOH and HFD model of pancreatitis. The results showed that MENS reduced oxidative stress and hence inflammation and also maintained the antioxidants level in pancreas.

CONCLUSION

MENS act as pancreato protective agent probably by reducing the prematured activation of cytokines and the production of ROS in a dose dependent manner. MENS minimizes the activation of proinflammatory cytokines by inhibiting the caspase-1 activation. MENS at the dose of 200 mg/kg body weight has a pronounced protective effect on EtOH and HFD induced pancreatic injury in rats. This study reveals that the optimum dose of 200 mg/kg body weight of MENS can be used for further study to evaluate the mode of pancreato protective action in rat model.

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