

Apigenin and Losartan Combinations Attenuate Monosodium Glutamate-Induced Changes in the Rat Exocrine Pancreas: Histological and Immunohistochemical Study

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Abstract

Background: Monosodium glutamate is an important food additive used as a flavor enhancer. In spite, many investigations showed its toxicity to the human and animals. Apigenin is a natural flavonoid with antioxidant, anti-inflammatory, and anti-proliferation properties. Losartan is Ang II (AT1) receptor blocker, used as an anti-hypertension and antifibrotic agent. The aim is to investigate apigenin and losartan protection separately and in combinations against monosodium glutamate-induced changes in the rat exocrine pancreas using histological and immunohistochemical studies.

Materials & Methods: 70 Wistar rats used, divided into 7 groups (10 rats each); Group 1: served as control, Group 2 (MSG): rats received monosodium glutamate 2 mg/g body weight/day in drinking water, Group 3 (Api): rats received apigenin 50 µg/rat/day, Group 4 (LST): rats received losartan 10 mg/kg/day, Group 5 (MSG+Api): rats treated with Api 50 µg/rat/day 30 min before MSG, Group 6 (MSG+LST): rats treated with LST 10 mg/kg/day 30 min before MSG and Group 7 (MSG+Api+LST): rats treated with Api and LST 50 µg/rat/day and 10 mg/kg/day respectively 30 min before MSG.

Results: MSG group showed marked acinar cell damage, apparently dilated ducts, inflammatory cellular infiltration, congested blood vessels with intimal loss and dissolution of the internal elastic lamina. These besides, significantly increased collagen fibers, significant decrease in PCNA positive acinar cell nuclei and lost E-cadherin expression. Conclusion: Apigenin and losartan combination attenuate MSG damaging effects on the exocrine pancreas. This is through their antioxidant, anti-inflammatory as well as antifibrotic effects.

Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid; a non-essential amino acid produced naturally in the organs and tissues of the human body playing an essential role in metabolism. Glutamate occurs naturally in high levels in meat, fish, poultry, and breast milk, while vegetables contain higher levels of free glutamate, especially peas, tomatoes, and potatoes. The free form of glutamate has a flavor-enhancing effect, so considered as an important additive added to foods, especially meats, poultry, seafood, snacks, soups and stews [1]. Nowadays, MSG is produced commercially through a natural fermentation procedure using molasses from sugar cane or sugar beets, as well as starch and corn sugar [2].

We consume MSG in our daily diet, either as a naturally occurring part of the foods that we eat or as a food ingredient [3]. In spite of its taste stimulation and improved appetite enhancement, many investigations showed that MSG is toxic to the human and animals. It can lead to several side effects, the most famous is the symptom complex known as Chinese restaurant syndrome (CRS); represented by vascular headache, edema, numbness, tingling, flushing, muscle tightness, and generalized weakness [4]. Moreover, MSG in high doses produces opposing systemic reactions comprises; hypertension, angina, arthritis, depression, neuroendocrine abnormalities, and neuronal degeneration [5], in addition to oxidative damage of the pancreas [2].

Apigenin (4', 5, 7-trihydroxyflavone) is a natural occurring flavonoid gained considerable attention as a beneficial compound for human health with cancer prevention and/or therapeutic properties [6]. Apigenin exists abundantly in fruits, such as oranges and grapefruit; plant-derived beverages, such as tea; vegetables, like parsley and onions; in addition to chamomile, wheat sprouts, and some seasonings [7]. Apigenin possesses an antioxidant, anti-inflammatory, anti-allergic and anti-proliferation properties, so it can protect many organs from repeated injuries [8]. Additionally, it has a potential role in cancer prevention and treatment, as it suppresses cell growing of many

human cancer cell lines, such as breast, colon, leukemia, skin, besides prostate cancer cells [9].

The renin-angiotensin system (RAS) is a circulating hormonal system producing physiologically active angiotensin II (Ang II) that regulates blood pressure, fluid and electrolyte homeostasis [10]. Many reports explored that pancreas exhibited its own RAS products like renin mRNA, angiotensinogen mRNA, Ang II and Ang II receptors type 1 (AT1) and type 2 (AT2) [11]. These are immunohistochemically localized in epithelial cells of the pancreatic ducts, endothelial cells of pancreatic blood vessels and acinar cells [12]. Conversely, RAS plays an important role in the pathophysiology of pancreatic injury and fibrosis through the overexpression of its products. Hence, by blocking Ang II receptors; the pancreatic tissue injury and fibrosis could be attenuated [13].

Losartan is one of the RAS inhibitors used for treatment of hypertension [14]. In addition to its antihypertensive effects, many studies demonstrated its potency as an antifibrotic agent [15]. Therefore, losartan could reverse the fibrogenesis process of pancreas attenuating its inflammation as well as fibrosis.

Accordingly, the aim of the current work is to investigate apigenin and losartan protection, separately, in combinations against monosodium glutamate-induced changes in the rat exocrine pancreas by histological, and immunohistochemical study.

Materials & Methods

Drugs and chemicals

Monosodium glutamate (Pharaana Chemical Co., Tanta, Egypt); Apigenin (Sigma Chemical Co., St. Louis, MO, USA); Losartan (Pharaonia Pharmaceuticals Co., Egypt); 0.5 % carboxymethyl cellulose (0.5% CMC), phosphate buffer, phosphate-buffered saline, hydrogen peroxide (El Gomhuria Chemical Co., Egypt); anti-E-cadherin antibody (Abcam, UK); anti- PCNA antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA); biotinylated goat anti-polyvalent antisera (Dako North America, Inc., CA, USA).

Animals

The present study was performed using 70 Wistar rats 150- 200 gm, obtained from the animal housing, Tanta University. They kept in plastic cages at $22 \pm 1^\circ\text{C}$ with $60 \pm 5\%$ relative humidity, fed on a standard laboratory diet and given water *ad libitum*. Animals handled according to the instruction for the care and use of experimental animals in Tanta University with an authorization of the Animal Experiment Committee of the University.

The experimental design

Rats divided into 7 groups, each contained 10 rats. Group 1 (control): further subdivided into 2 subgroups; the first one kept without treatment and the second one received 0.5% CMC. Group 2 (MSG): rats received MSG in a dose of 2 mg/g body weight/day in drinking water for 30 days [16]. Group 3 (Api): rats received apigenin orally in a dose of 50 $\mu\text{g}/\text{rat}/\text{day}$. Group 4 (LST): rats received losartan orally in a dose of 10 mg/kg/day. Group 5 (MSG+Api): rats treated orally with Api in a dose of 50 $\mu\text{g}/\text{rat}/\text{day}$ 30 min before MSG for 30 days [17]. Group 6 (MSG+LST): rats treated orally with LST in a dose of 10 mg/kg/day 30 min before MSG for 30 days [12]. Group 7 (MSG+Api+LST): rats treated orally with Api and LST in a dose of

50 $\mu\text{g}/\text{rat}/\text{day}$ and 10 mg/kg/day respectively 30 min before MSG for 30 days.

- One day after the last dose, rats anesthetized via injection of 60 mg/kg pentobarbital intraperitoneally. An abdominal midline incision made and the pancreas taken out and processed for histological and immunohistochemical examination.

Preparation of specimens for light microscopic examination

The extracted pancreatic specimens processed through the following steps. In brief, specimen's directly fixed in 10% formalin buffered saline for 3 days, dehydrated in ascending grades of alcohol, cleared in two changes of xylene, and then embedded in hard paraffin. Afterward, sections of 5 μm thick obtained and stained for histological and immunohistochemical examination [18,19].

Stains used for histological examination:

Hematoxylin and Eosin stain (H. & E.): Briefly, sections deparaffinized, hydrated, and then stained with hematoxylin and eosin. Followed by dehydration, clearing and mounting in Canada balsam. In H. & E. stained sections, pancreatic histopathology for each animal examined and scored based on acinar cell damage, duct dilatation, blood vessel's congestion, and interstitial inflammatory cell infiltration. Then, each parameter quantified and scored using a random scale ranging from 0 to 3 as follows: 0 = none, 1 = mild, 2 = moderate and 3 = sever changes.

Mallory's trichrome stain: Tissue specimens immersed in saline, then in Bouin's fluid. Followed by staining with trichrome solution, followed by rapid dehydration, clearance and mounting. After that, evaluation of the area percentage (area %) of pancreatic collagen content in the septa between pancreatic lobules, around acini, ducts and blood vessels done by using ten readings from ten non-overlapping sections of each rat at magnification X100 through Leica Q500 image analyzer (Pathology Department, Faculty of Medicine, Tanta University, Egypt). The area percentage and the standard measuring frame of a standard area was equal to 118 476.6 μm^2 .

Elastica Van Gieson's (Verhoeff's Van Gieson's): In brief, sections deparaffinized and hydrated, followed by adding Verhoeff's hematoxylin for 30 min. Sections then differentiated in 2% ferric chloride solution and counterstained with Van Gieson's for 5 minutes. Finally, sections dehydrated, cleared, and mounted.

Immunohistochemical localization of epithelial cadherin (E-cadherin) & proliferating cell nuclear antigen (PCNA): Pancreatic specimens deparaffinized, hydrated, and then placed in 0.3% hydrogen peroxide/methanol for 20 min to inactivate endogenous peroxidase activity. After immersion in 10 ml of citrate buffer (pH=6), sections autoclaved for 10 minutes (to expose the antigen) then left to cool at room temperature. Next, the specimens treated with a serum-free protein blocking solution and incubated 60 min at 4°C with a mouse monoclonal anti-E-cadherin (1:100 dilution) & anti- PCNA antibodies (1:100 dilution). Afterward, specimens incubated with polyvalent anti-mouse secondary antibody and 1 - 2 drop of DAB applied. After counterstaining with Mayer's hematoxylin, sections dehydrated, cleared, mounted, and examined via light microscope (Olympus, Japan). For the negative control, PBS added instead of the primary antibody.

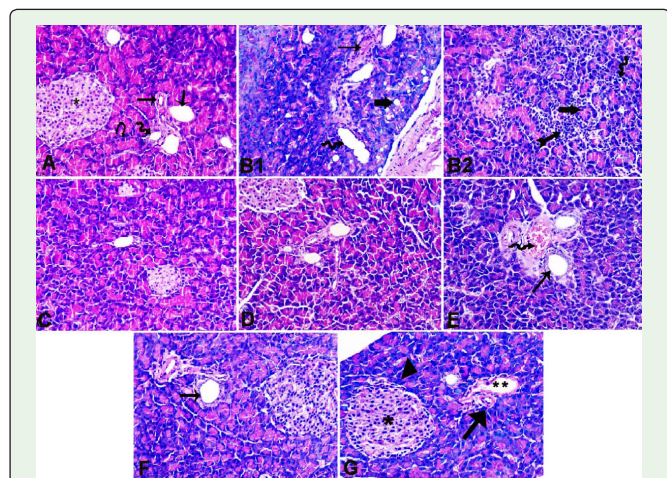


Figure 1: Effect of Apigenin and Losartan on H & E stained sections of monosodium glutamate-induced changes in the rat exocrine pancreas: (A) Control: showed pancreatic lobules, thin C.T. containing blood vessels (→), and ducts (wavy arrow), pyramidal acinar cells with apical acidophilic cytoplasm and basal rounded nuclei (curved arrow), and islets of Langerhans (°). (B1,2) MSG: showed apparently dilated ducts lined with flattened epithelium (wavy arrow), congested blood vessels (thin arrow), acinar cell cytoplasmic (thick arrow), and perinuclear vacuolation (wavy arrow), darkly stained nuclei (thick arrow) and interstitial mononuclear cellular infiltrations (tailed arrow). (C) Api: like control. (D) LST: like control. (E) MSG+Api: showed apparently dilated ducts (→) and congested blood vessels (wavy arrow). (F) MSG+LST: showed apparently dilated ducts (→). (G) MSG+ Api+LST: pancreatic lobules separated by a thin connective tissue containing blood vessels (→), and ducts (°), acini with pyramidal acinar cells possessed apical acidophilic cytoplasm and basal rounded nuclei (↗), and islets of Langerhans (°) (H&E X 400).

Additionally, number of acinar cells positive for PCNA counted in 10 randomly selected fields by using a light microscope (Olympus, Japan) at a magnification X 400. The PCNA-labeling index represented the percentage of PCNA-positive cells per total number of acinar cells counted.

Statistical analysis

The achieved data subjected to statistical analysis using the Minitab Statistical Software for Windows (version 16.1, Minitab Inc., USA). Variances then analyzed by the two-tailed Student's t-test after evaluation of F-test. Data expressed as mean ± standard deviation through which the results considered statistically significant, when P value < 0.05.

Results

- In view of group 3 (Api) and group 4 (LST), their results were similar to the control group.

H. & E.

The examined control group revealed, pancreatic lobules separated from each other by a thin connective tissue containing blood vessels, and ducts. The lobules formed of closely packed acini having pyramidal acinar cells with apical acidophilic cytoplasm and basal rounded nuclei. In addition, islets of Langerhans appeared within the exocrine pancreatic tissue as pale oval areas with groups of cells separated from each other by blood capillaries. In comparison to the control group, MSG group showed apparently dilated ducts lined with flattened epithelium, besides congested blood vessels. Furthermore, the acini were irregularly arranged, widely separated with interstitial mononuclear cellular infiltrations. Concerning the acinar cells, they exhibited cytoplasmic and perinuclear vacuolation, in addition to darkly stained nuclei. The MSG+Api group exposed apparently dilated ducts and congested blood vessels. When seeing the MSG+LST group, apparently dilated ducts observed. Moreover, pancreatic architecture in MSG+ Api+LST group revealed pancreatic lobules separated by a thin connective tissue containing blood vessels, and ducts. The lobules formed of acini with pyramidal acinar cells possessed apical acidophilic cytoplasm and basal rounded nuclei. Besides islets of Langerhans (Figure 1 and Table 1).

Mallory's trichrome

The collagen fibers of the control group seen as delicate fibers in-between the pancreatic lobules, acini, as well as around ducts and blood vessels. For MSG group, apparently increased collagen fibers, especially around the dilated ducts and congested blood vessels noticed. Seeing the MSG+Api group, relatively moderate amount of collagen fibers observed to be only little ones around the apparently dilated ducts in MSG+LST group. Considering MSG+ Api+LST group, a picture similar to the control group could be seen. By which delicate fibers in-between pancreatic lobules, acini, around ducts, and blood vessels were observed (Figure 2).

With regard to the mean area% of pancreatic collagen content, a significant increase in MSG group detected in contrast to the significant decrease in the treated groups MSG+Api, MSG+LST, MSG+Api+LST when compared to control and MSG group respectively. For Api and LST groups, no significance observed in comparison to the control group (Figure 3).

Verhoeff's Van Gieson

Through examination of the control group, intact blood vessels with intact intimal lining having a distinct well defined internal elastic lamina detected. Conversely, thickened wall of the blood vessels in addition to intimal loss and dissolution of the internal elastic lamina seen in MSG group. In MSG+Api group, areas of intimal loss with the focal dissolution of internal elastic lamina detected. While in MSG + LST an area of internal elastic lamina, dissolution noticed.

Table 1: Histopathological scoring in H. & E. stained sections of the different experimental groups.

	Control	API	LST	MSG	MSG+API	MSG+LST	MSG+API+LST
Acinar cell damage	0	1	0	3	1	1	0
Ducts' dilatation	0	0	0	3	2	2	1
Blood vessel's congestion	0	0	0	3	2	0	0
Interstitial inflammatory cellular infiltrations	0	0	1	3	0	0	0

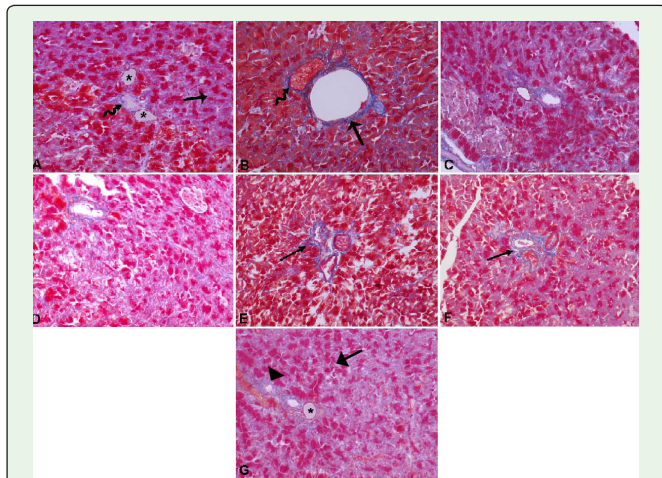


Figure 2: Effect of Apigenin and Losartan on monosodium glutamate induced changes on the collagen fibers stained by Mallory's trichrome. (A) Control: showed delicate collagen fibers in-between the pancreatic acini (→), around ducts (wavy arrow) and blood vessels (*). (B) MSG: showed apparently increased collagen fibers, especially around dilated ducts (→) and congested blood vessels (wavy arrow). (C) Api: like control. (D) LST: like control. (E) MSG+Api: showed relatively moderate amount of collagen fibers (→). (F) MSG+LST: showed little fibers around dilated ducts (→). (G) MSG+Api+LST: revealed delicate fibers in-between acini (→), around ducts (>), and blood vessels (*). (Mallory's Trichrome X 400).

In MSG+Api+LST, nearly normal blood vessel's appearance seen with normal intimal lining and well defined internal elastic lamina (Figure 4).

E-cadherin

The control group exhibited a strong membranous expression of E-cadherin and some cytoplasmic reaction in pancreatic acini. By observing MSG group, lost membranous expression in most of pancreatic acini found. Considering MSG+Api group, reduced membranous expression in some acini observed, while in MSG+LST group partial loss of the membranous E-cadherin expression of few acinar cells experienced. On the other hand, MSG+ Api+LST group, showed a reaction similar to the control group (Figure 5).

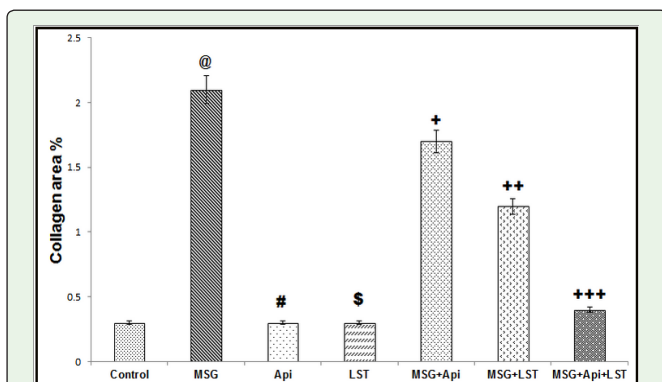


Figure 3: The mean are a percentage of pancreatic collagen content expressed as mean ± SD. MSG group showed significant increase in comparison to the control group. Significant decrease in the treated groups MSG+Api, MSG+LST, and MSG+Api+LST when compared to MSG group. Api and LST groups, revealed no significance in comparison to the control group. @ $P < 0.05$; # & \$ $P > 0.05$; +, ++, +++ $P < 0.05$.

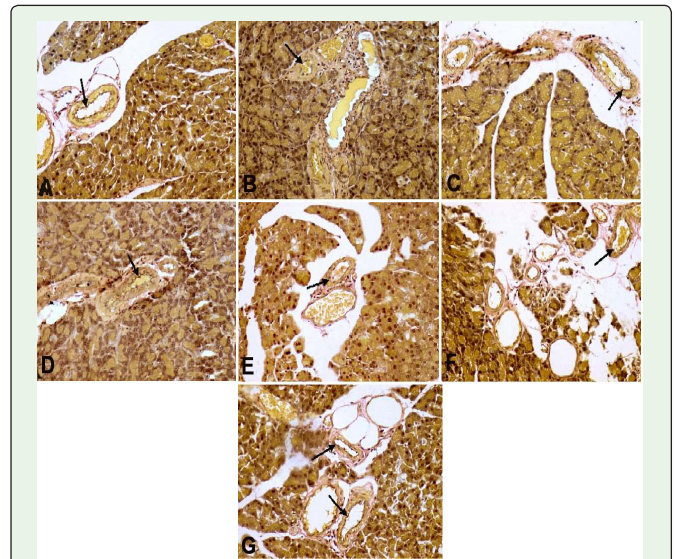


Figure 4: Effect of Apigenin and Losartan on monosodium glutamate induced changes on the pancreatic blood vessels stained by Verhoeff's Van Gieson. (A) Control: showed intact intimal lining blood vessels and distinct well-defined internal elastic lamina (→). (B) MSG: showed thickened wall of the blood vessels, intimal loss, and dissolution of the internal elastic lamina (→). (C) Api: similar to control with normal intima (→). (D) LST: like control with normal intimal lining (→). (E) MSG+Api: showed areas of intimal loss with focal dissolution of internal elastic lamina (→). (F) MSG+LST: showed an area of internal elastic lamina dissolution (→). (G) MSG+Api+LST: Exposed nearly normal blood vessel's appearance with intact intima and internal elastic lamina (→). (Verhoeff's Van Gieson X 400).

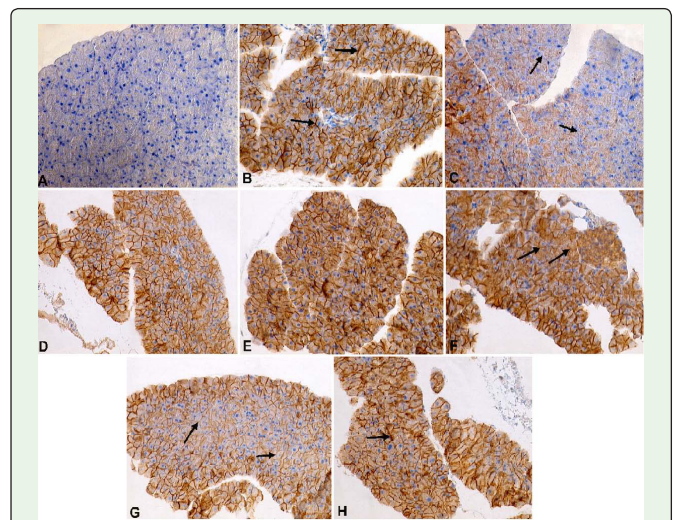


Figure 5: Effect of Apigenin and Losartan on monosodium glutamate induced changes on pancreatic E-cadherin expression. (A) Negative control without primary antibody. (B) Control: revealed strong membranous expression of E-cadherin and some cytoplasmic reaction in pancreatic acini (→). (C) MSG: revealed lost membranous expression in most of pancreatic acini. (D) Api: like control. (E) LST: like control. (F) MSG+Api: revealed reduced E-cadherin membranous expression in some acini (→). (G) MSG+LST: revealed partial loss of the membranous E-cadherin expression of few acinar cells (→). (H) MSG+Api+LST group: showed strong membranous expression of E-cadherin in similarity to the control group (→). (E-cadherin X 400).

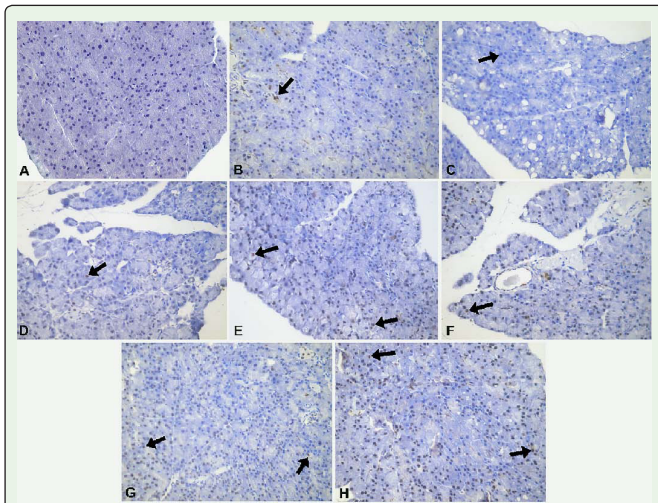


Figure 6: Effect of Apigenin and Losartan on monosodium glutamate induced changes on pancreatic PCNA expression. (A) Negative control without adding primary antibody. (B) Control: expressed few acinar cells with positive PCNA reaction (→). (C) MSG: revealed absent PCNA-positive nuclei (→). (D) Api: similar to control with positive PCNA reaction of few acinar cells (→). (E) LST: few acinar cells with positive PCNA reaction like control (→). (F) MSG+Api: showed some acinar cells with positive nuclear reaction (→). (G) MSG+LST: showed some acinar cells with positive nuclear reaction. (H) MSG+Api+LST: revealed majority of acinar cells with PCNA-positive nuclei (→). (PCNA X400).

PCNA

Examination of the control group for PCNA nuclear reaction, exposed few acinar cells with a positive reaction. In MSG group, absent PCNA-positive nuclei obviously seen. While in MSG+Api as well as MSG+LST groups, some acinar cells with positive nuclear reaction observed. Regarding MSG+ Api+LST group, most of the acinar cells with PCNA-positive nuclei obviously seen to be similar to the control group (Figure 6).

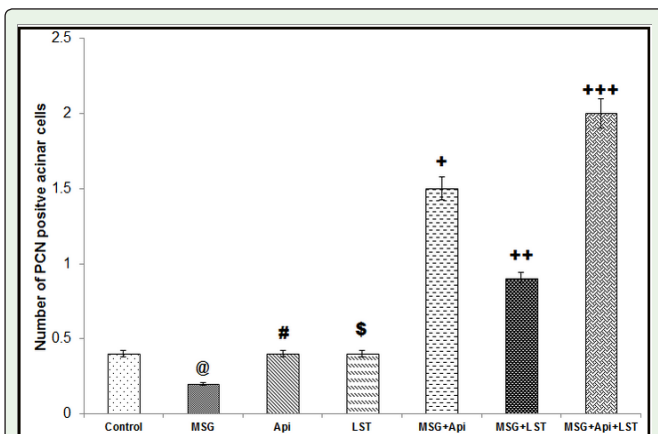


Figure 7: The number of acinar cells positive for PCNA expressed as mean ± SD. MSG group showed significant decrease in PCNA positive cells in comparison to the control. Whereas, significantly increased expression of nuclear PCNA observed in MSG+Api, MSG+LST, and MSG+Api+LST when compared to MSG group. Moreover, there was no significance in Api and LST groups when compared to control. @ $P < 0.05$; # & \$ $P > 0.05$; +, ++, +++ $P < 0.05$.

In view of the number of PCNA acinar cell positive nuclei, MSG group showed significant decrease in comparison to the control group. Whereas significantly increased expression of nuclear PCNA observed in MSG+Api, MSG+LST, as well as MSG+Api+LST when compared to MSG group. Additionally, there was no significance in Api and LST groups when compared to control (Figure 7).

Discussion

The present study evaluated the possible protective effect of Api and LST each alone as well as in combinations on MSG-induced changes on the rat exocrine pancreas. Our results showed that MSG-induced histopathological degenerative changes in the pancreatic acini. Besides, significant increase in collagen fibers, and thickened wall of the blood vessels along with intimal loss and dissolution of the internal elastic lamina. Moreover, there were lost membranous expression of E-cadherin besides significantly increased acinar cell PCNA-positive nuclei. Additionally, the treated groups with both Api and LST provided better amelioration of the previous histological and immunohistochemical findings than that treated with either Api or LST alone.

MSG is a worldwide food additive added to food as a flavor enhancer [3]. In the present research, MSG produced marked histopathological changes in the pancreatic acini. This in parallel to the results recorded by Leshchenko et al. (2012) [20] who proved that long-term administration of MSG produce degenerative changes in the exocrine cells, cellular infiltrations, fibrosis, and edema.

MSG acts through alteration of the ionic permeability of the neural membranes, leading to depolarization. Which is an excitatory neurotransmitter playing an important role in different pancreatic pathological processes [2]. MSG chronic administration lead to hyperglutamatemia with an adverse effect on the hypothalamus destroying its cells, thus, impairs leptin and insulin signaling resulting in hyperleptinemia and hyperinsulinemia with obesity [21]. Additionally MSG-induced obesity leads to increased activity of the exocrine pancreatic lipase that mediates the hydrolysis of circulating triglycerides (TG). These are injurious to the acinar cells as they activate trypsinogen with subsequent local autodigestion of pancreatic tissue in addition to capillary plugging and localized ischemia. Thus, pancreatitis, pancreatic insufficiency, and necrosis occurs, leading to increased pancreatic cell damage and turnover [21,22].

Thomas et al. (2009) [23] added that, MSG induced changes in the mitochondrial lipid peroxidation as well as its antioxidant status in different regions of the brain with subsequent hyperphagia and obesity. Consequently, this led to lipogenesis and hyperlipidemia. These metabolic alterations resulted in lipid peroxidation of the biomembranes and production of oxygen free radicals causing membranes oxidative stress damage, cellular dysfunction along with tissue damage and injury [24]. Similar observations also verified in researches involving MSG toxic effect on other organs like kidney and liver [25,26].

In our study, the mean area percentage of collagen significantly increased in the MSG group in comparison to the control. These results also in agreement with Yin et al. (2013) [27]. It may occur as a physiological healing process in response to the pancreatic tissue damage and injury induced by MSG [16].

Moreover, loss of E-cadherin expression by MSG explained by the action of glutamate on its peripheral pancreatic receptors inducing membrane depolarization besides increased intracellular calcium level [28]. Subsequently, this increased calcium level will enhance the metalloprotease-mediated E-cadherin cleavage with degradation of its cytoplasmic domain resulting in translocation of β -catenin from the cell membrane to the cytoplasm [29].

Additionally, the present study indicated the decreased number of PCNA positive acinar cell nuclei of MSG group when compared to the control. These findings were consistent with that found by Nakayama et al. (2003) [22] during their study on MSG effects on the pancreas. They attributed these changes to the damage of pancreatic acinar cells as well as decreased pancreatic DNA content caused by long-term ingestion of MSG.

The congested and thickened wall of the blood vessels as well as intimal loss and dissolution of the internal elastic lamina noticed in MSG group. MSG possesses a vasoconstrictive effect on the blood vessels as a calcium channel opener, so a change in its diameter ensues. These results correspond with the results conveyed by Ismail (2012) [30] who revealed congestion of the testicular blood vessels after treatment of rats with MSG. Moreover, pancreas uptakes glutamate leading to reversal of the mitochondria malate/aspartate transport and concurrently the proton pump. This consecutively causes a decrease of cytosolic pH and increased release of bound calcium from its intracellular stores like smooth endoplasmic reticulum, and mitochondria resulting in tissue anoxia [31].

The present study also disclosed increased cellular infiltration in MSG group. This explained by the increased TNF- α and IL-6 mRNA gene expression by MSG [32]. The over-production of pro-inflammatory cytokines let organisms to respond to infectious agents inducing inflammation and cellular infiltration [33]. Furthermore, many studies revealed that obesity associated with chronic MSG administration leading to a low-grade inflammation. Through which visceral adipose tissue stimulated to synthesize and secrete cytokine and adipokines like IL-1 β , IL-6, and TNF- α , resistin, adiponectin as well as leptin. Besides MSG increased IL-1 β and decreased IL-10 levels in serum, leading to a reduction in the ratio between circulating anti- and pro-inflammatory cytokines [34].

Apigenin (4, 5, 7-trihydroxyflavone) is a flavonoid present in herbs such as chamomile. Different studies indicated that, apigenin possesses anti-inflammatory and anti-proliferation properties [7]. It exerts its pancreatic protection through reduction of the pro-fibrosis (collagen 1A1 and fibronectin) and pro-inflammation (IL-6 and IL-8) mRNAs production in pancreatic stellate cells (PSCs), as well as inhibition of PSCs proliferation and induction of their apoptosis [17]. These PSCs when activated; becomes myofibroblast-like cells with the production of extracellular matrix proteins (collagen and fibronectin) and cytokines, leading to inflammation and fibrosis of the pancreas [35]. Therefore, thru their inhibition by apigenin, the pancreatic histopathological findings induced by MSG ameliorated.

Apigenin known as a potent antioxidant, that can act in hydrophilic and hydrophobic environments rather than other antioxidants that act only in hydrophobic systems. It works through inhibiting the biomolecules from undergoing oxidative damage by scavenging reactive oxygen species, inhibition of enzymes, chelation of metal ions like Fe³⁺, and Cu⁺ (which catalyze various processes leading

to free radical appearance), promotion of free radical production, inhibition of lipid oxidation and regeneration of membrane-bound antioxidants such as R-tocopherol [36].

Additionally, apigenin attenuate inflammation through inhibition of cyclooxygenase-2 and 5-lipoxygenase, which involved in the metabolisms of arachidonic acid, subsequently prostaglandin release is inhibited [37]. Moreover, apigenin inhibit cytokine-induced adhesion protein expression in endothelial cells as well as it augments apoptosis of recurrently activated human T cells, by interfering in both extrinsic and intrinsic pathways [8].

The present study showed improvement of E-cadherin expression in the apigenin treated group. This may attributed to the increased protein levels of E-cadherin by apigenin as well as the inhibition of the nuclear translocation of β -catenin and its retention in the cytoplasm [6].

The present work exposed that apigenin does not improve the congestion of blood vessels. This may due to interference with collagen-stimulated platelet function by blockage of Fyn kinase activity and the tyrosine phosphorylation of Syk and PLCg2 in a megakaryocytic cell line. Moreover, apigenin does not interfere with thrombin receptors, but weakens platelet aggregation through signaling pathways that inhibit kinase activation [38].

Many studies have recorded the role of the local pancreatic RAS in the fibrogenesis process as well as the antifibrogenic effect of treatments with AT1blockers or Angiotensin-Converting Enzyme (ACE) inhibitors [39]. Losartan is an AngII (AT1) receptor inhibitor [10]. It inhibits PSCs DNA synthesis through AT1 receptor, result in inhibition of their contraction, proliferation, and migration with the inhibition of collagen deposition in the ECM, and so reverse the process of pancreatic fibrosis [40]. Diop-Frimpong et al. (2011) [15] added that losartan exerts its antifibrotic activity by reducing the levels of collagen type I and type III as well as collagen IV present in the basement membranes that is mediated by the suppression of TGF- β 1-dependent and independent pathways via AT1 receptor downregulation.

In the present study, the anti-inflammatory effect of losartan may attributed to its inhibition of monocyte chemoattractant protein-1 (MCP-1), IL-8, TNF- α and IL-6 production that mediated through AT1 [41], so prevent infiltration of neutrophils in addition to reducing of cytokine release in the pancreas [42]. Furthermore, losartan increases the plasma levels of adiponectin, which is an adipose-specific protein that increase insulin sensitivity and inhibit inflammation [43]. Additionally, losartan counteracts the action of AT1 on blood vessels by decreasing the intracellular Ca²⁺ as well as the contractile force of vascular smooth muscle cells [44] inducing vasodilatation and decreased the sodium and water re-absorption [39]; improving the pancreatic microcirculation with regeneration of the acinar cells [45].

The present study revealed that losartan increased acinar cells PCNA expression. This can be explained by the inhibitory effect of losartan on acinar cell apoptosis by blocking AT1 receptors during the development of pancreatic fibrosis. This action is accompanied with the down-regulation of apoptosis-linked genes, such as Bax, Bak, as well as Bcl-2 mRNA, favoring regeneration and proliferation of acinar cells [46].

The restoration of E-cadherin expression by losartan in the present research; may be due to TGF- β 1 downregulation by losartan inhibiting its expression and activation [15]. Furthermore, the study made by Arnold et al. (2012) [47] on pancreatic tumors revealed that losartan abrogated TGF- β 1-induced gene expression with up-regulation of E-cadherin expression.

Conclusions

The present research showed that MSG has damaging effects on the exocrine pancreas and the simultaneous administration of both apigenin and losartan attenuate such effects through their antioxidant, anti-inflammatory as well as antifibrotic effects. Therefore, safety doses of MSG as a flavor enhancer should be assessed and monitored. In addition, individuals ought to limit their dietary consumption of foods containing MSG.

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