ISSN:0975-5772

Gamel El-Din I.Harisa / Journal of Pharmaceutical Science and Technology Vol. 2 (2), 2010, 139-147

Effects of aminoguanidine on some biochemical changes associated with acetic acid -induced ulcerative colitis in rats

Gamal El-Din I. Harisa

Department of Biochemistry, College of Pharmacy (Boys), Al-Azhar University,
Nasr City, Cairo, Egypt

E-mail: g_harisa@yahoo.com

Abstract:

Inducible nitric oxide has been implicated the pathogenesis associated with ulcerative colitis. Moreover oxidative stress linked with ulcerative colitis is responsible for inactivation of many antioxidants enzymes. The aim of the present study is to investigate the protective effect of aminoguanidine against acetic acid-induced ulcerative colitis in rats. In this study the effects of aminoguanidine1g/l supplementation to the rats on some biochemical markers for oxidative stress were assessed. In addition, the colonic tissues macroscopic and microscopic changes were investigated. The results of present study indicated that intra-rectal inoculation of the rats with 4% acetic acid for 3 consecutive days revealed a significant alteration of macroscopic, microscopic as well as increase of colon weight and decrease colon length. Aminoguanidine administration was significantly ameliorating the degeneration caused by acetic acid inoculation. Pretreatment of rats with aminoguanidine preserve the activity of serum paraoxonase by about (36%), and colon tissues catalase (144%), moreover aminoguanidine treatment elevate reduced glutathione tissues colon content by 267 % compared with acetic acid administration. Serum nitrate level, colonic tissue content of malondialdehyde and protein carbonyls were reduced by aminoguanidine prior treatment by 175%, 47% and 60 % respectively compared with ulcerative colitis rats. Finally we concluded that increase of nitric oxide plays an important role in the pathogenesis of ulcerative colitis. This resulted in decrease of colonic tissues reduced glutathione contents as well as reduction of some antioxidant enzymes activities. Restoration of nitric oxide level by aminoguanidine treatment is responsible for attenuation of oxidative stress caused by acetic acid administration. Our results suggested that aminoguanidine treatment can reduce the inflammation associated with ulcerative colitis as confirmed with histopathological investigations.

Keywords: *ulcerative colitis, aminoguanidine, paraoxonase-1, malondialdehyde, protein carbonyl, nitrate*

Introduction

Oxidative stress have been implicated in the pathogenesis of a diversity of acute and chronic inflammatory diseases such as atherosclerosis, rheumatoid arthritis and ulcerative colitis (UC)[1]. This lead to extensive efforts to define the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the inflammatory process along with stimulated the search for agents that may affect the production and bioavailability of ROS and RNS [2].

UC is associated with neutrophils infiltration and increase production of superoxide through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase as well as the upregulation of the inducible nitric oxide synthase (iNOS) [3]. The increase of superoxide and nitric oxide (NO) in UC was resulted in the increase of peroxynitrite formation which mediates oxidation of lipids, protein and DNA [4]. Moreover when the production of both ROS and RNS exceeds the capacity of the biological antioxidant defenses to detoxify them; the oxidative damage of the cells was occurs [5].

Antioxidant enzyme systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase in addition to reduced glutathione (GSH) as non enzymatic antioxidants provide the protection against oxidative damage produced by either ROS or RNS [6] .Paraoxonase-1 (PON1) is one of an antioxidant enzyme present in the serum on high-density lipoprotein (HDL) that hydrolyses lipid peroxides [7]. Furthermore, PON1 protects low density lipoprotein (LDL) and HDL from oxidation induced by free radical [8]. PON1 activity has been suggested to be inversely associated with oxidative stress in serum and macrophages [9]. Also, PON1 activity was decreased in many inflammatory conditions, such as rheumatoid arthritis [10], age-related degeneration [11], steatohepatitis [12] and Behcet.s disease [13].

However UC is associated with increase of the activity of iNOS and augmentation of

NO production [14], the use of iNOS inhibitors has substantial effect in the protection against tissues damage in this case [15]. Among of these inhibitors aminoguanidine (AG), this is known to be a selective inhibitor of iNOS [16], moreover AG was reduce the rate of free radical production and minimize the biological oxidative damage [17]. In this study UC was induced in rats by intra-rectally administration of 4% acetic acid (AA). The effects of AG supplementation on lipid profile and some biochemical markers for oxidative stress such as the activities of paraoxonase, superoxide dismutase and catalase, as well as levels of nitrate, proteins carbonyls (PCO) as marker for protein oxidation and malondialdehyde (MDA) as marker for lipid peroxidation were assessed. In addition, colonic tissues macroscopic and microscopic changes were investigated.

Materials and Methods:

Material

Aminguanidine, paraoxon, GSH, SOD, CAT, Ellman's reagent ,thiobarbituric acid , NADPH, FAD, nitrate reductase, and tetraethoxy-propane were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were available of the highest analytical grade.

Instruments and apparatus:

Beckman XL-70 ultracentrifuge, 100,000 rpm (USA). Spectrophotomètre SHIMADZU (UV-1201 UV-VIS) (Japan). Homogenizer: Janke and Kunkel IKA 8,000 - 20.500 rpm (Germany).

Animals

Male Sprage-Dawely rats, weighing 220 – 250 g were obtained from locally bred strain and housed 4 per cage in our animal facility for 15 days before the binging of experimentation. Animals were fed on standard diet pellets (El-Nasr Co. Abou-Zaabal, Egypt) and housed under a 12 h light/dark cycle at a constant temperature (22 ± 2 ºC), with food and water *ad libitum*.

Induction of ulcerative colitis

Before the induction of UC, the rats were deprived of food, but not water, for 18 hour. The generation of UC was performed by intracolonic administration of 2 ml of 4% (v/v) AA in saline (pH 2.3) through a polyethylene tube (PE-60), the tip of which was positioned in the colon 8 cm past the anus under effect light ether anesthesia. AA was slowly administered into the colonic lumen, after a 30 second period of exposure, excess fluid was withdrawn, and the colon was then flushed with 1.5 ml of phosphate-buffered saline (pH 7.4). Control animals were subjected to the same procedure with the exception that isotonic saline was substituted for AA[18].

Experimental Protocol

Twenty four rats were randomly divided into 3 groups 8 animals in each group. In control group rats were received isotonic saline intrarectally while in rats in UC group were inoculated intra-rectally with 2 ml/day of 4% AA in isotonic saline for three days. In AG treated group rats were received AG bicarbonate *ad libitum* in drinking water at a concentration of 1 g/L for 4 weeks before intrarectally inoculation with 4% AA for 3 days [19]. Experimental procedure was conducted according to the guidelines of institutional animal ethical committee of College of Pharmacy, Al–Azhar University, Nasr City, Cairo, Egypt.

Samples collection

Blood samples were drown from the ocular vein, collected into centrifuge tubes and left to stand at room temperature for 10 minutes, then centrifuged at 3000 rpm for 10 minutes. The serum were isolated and stored at -20°C until biochemical investigations. Then the animals were sacrificed by cervical decapitation under ether anesthesia, the abdomens were opened using a surgical scissor. Colon was removed via laparotomy, opened longitudinally, washed three times with cold phosphate-buffered saline pH 7.4 and the colons weights and lengths were measured. Moreover the colon stretched

on a piece of cork and examined macroscopically for assessment of ulcer index. Then colons tissue were homogenized with 10 ml of ice-cold 0.25 M sucrose and centrifuged at 14,000 rpm for 20 min and stored at -20°C till estimation of the tissues biochemical parameters [20].

Assessment of tissue injury

The distal 8 cm of colons were opened down their mesenteric borders and cleansed of luminal contents. The severity of gross macroscopic damage in the colon was then graded using the following criteria: 0, normal appearance; 1, focal hyperaemia, no ulcers; 2, single site of ulceration without associated inflammation; 3, single site of ulceration with inflammation 4, two or more sites of discrete ulceration and inflammation; 5, major site of injury or inflammation extending 1–2 cm along length of colon; and 6–10, score increased by one for each additional centimeter of damage or injury beyond 2 cm[21].

Biochemical measurements

Serum parameters

Serum level of total cholesterol (TC) [22], HDL–C[23], as well as triacylglycerol (TAG) [24] were determined based on previously described methods using a commercially available kit (Biocon DiagnostiK, Germany). LDL–C was calculated using Friede–wald equation [25].PON1 activity in was determined using paraoxon as substrate; the rate of hydrolysis of paraoxon was measured at 412 nm [26]. Nitrate level is measured by using of nitrate reductase method [27].

Colonic tissues parameters

PCO were determined by dinitrophenyl hydrazine (DNPH) assay. Briefly, colon tissues were homogenized in a lysis buffer, the insoluble cellular debris was removed by centrifugation. Aliquots in protein samples were precipitated with HCl-acetone and washed with trichloroacetic acid (TCA) solution. Pellets were re-suspended in the buffer solution and reacted with DNPH by vortexing for 15 min. To remove the un-reacted DNPH, the centrifuged pellets were washed with 20% TCA and 5 ml of ethanol: ethyl acetate mixture (v/v = 1:1). The final precipitate was resolved in 1 ml of 6 mol/l guanidine HCl, and the absorbance was measured spectrophotometrically at 380 nm [28].

Assay of SOD activity was based on the inhibition of pyrogallol auto-oxidation [29]. CAT activity was measured by the method based on the measurement of the decrease in hydrogen peroxide concentration at 240 nm using a spectrophotometer [30]. GSH content is determined as described by [31]. MDA is estimated by determination of the tissue content of thiobarbituric acid reactive substance [32]. The total protein concentration was measured according to the method of Lowry et al [33].

Histopathological investigations

Tissue specimens were taken from the colons of each of the 3 groups of rats and then fixed in 10% formalin saline solution for 24 hours. Trimming was done on the fixed tissue specimens and washed in tap water for 12 hours. Serial alcohols (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3 micron thickness by slide microtome. The obtained tissue sections were collected on glass slides and stained by heamatoxylin and eosin for histopathological examination by the light microscope [34].

Statistical analysis

Results were expressed as means ± standard error of the mean (SEM) and were analyzed for statistically significant differences using one–way analysis of variance (ANOVA) followed by the Tukey–Kramer post analysis test to compare all groups. P values less than 0.05 were considered significant. GraphPad Prism® was used for statistical calculations (Version 5.00 for Windows,

GraphPad Software, San Diego California USA).

Results:

Serum TC and LDL-C level of UC rats were elevated by about 39 % and 116% respectively while HDL-C was reduced by 32 % compared with control group .On another hand pretreatment with AG reduce serum TC by about 14% and elevate serum HDL-C by 31% regarding to UC group.

In respect to PON1 activity AA treatment produces reduction of serum PON1 by 33% compared with control rats, on another hand AG treatments prior to acetic acid significantly preserve serum PON1 activity by 36% as compared to acetic acid-induced UC. Furthermore serum nitrate level was significantly increased by AA-administration (163%) when compared with the control group. Conversely, in the group treated with AG serum nitrate levels is reduced by 175 % in relation to colitis group (Table 1).

Table (2) demonstrates the level of PCO, GSH, SOD and CAT in colon tissue in different studied groups. The rats colon content of PCO content as well as MDA content were significantly increased by 178%, 256% respectively in AA-inoculated rats in relation to control one. On the other hand, there are significant decreases of PCO and MDA colon level in group treated with AG by about 47% and 60 % respectively compared with UC group.

In the present study, there was a significant decrease in GSH colon content by AA administration (324%) compared to the control group. Pretreatment of rats with AG significantly maintain the colonic tissues GSH contents (267%) as compared to the colitis group. SOD activity in colonic tissues was significantly decreased in acetic acid-induced colitis group (83%) compared to the control group. In contrast, there were significant elevations in SOD activities in groups treated with AG (403%).Table (2) also indicated that AA inoculation significantly decreased CAT colonic activity by about 58% as compared to control group. On the other hand, AG significantly increased CAT activities to 144% as compared to AA -treated group.

Intra-rectal administration of 4 % AA to rats significantly increased the colon weight and markedly decreased the colon length amounting to 50 % and 24%, respectively. On the other hand, administration AG tend to moralize the alteration of colon weight and length caused by acetic acid (Figure1&2).

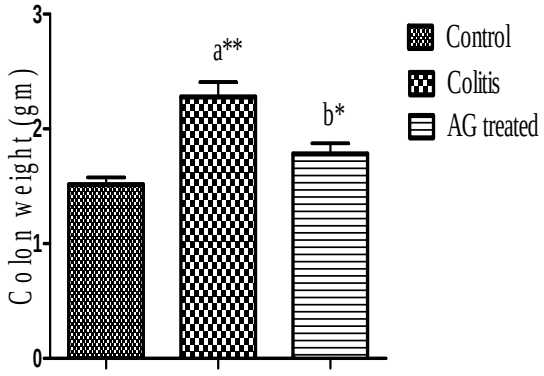

Figure 1: Effect of acetic acid inoculation and AG treatment on rat's colon weight (gm)
Data are represented as mean ± SEM of 8 rats per group
[a] Significantly different from control group
[b] Significantly different from colitis group
*P < 0.01 **P < 0.001

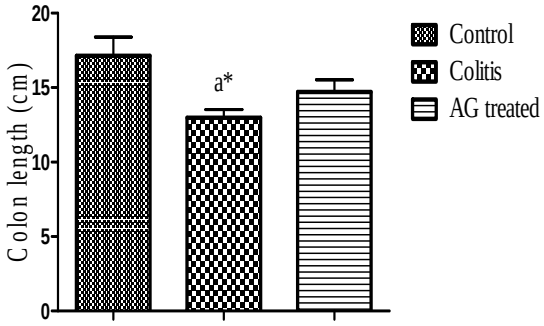

Figure 2: Effect of acetic acid inoculation and AG treatment on rat's colon length (cm)
Data are represented as mean ± SEM of 8 rats per group; [a] Significantly different from control group at *P < 0.01

Table 1: Serum TC, HDL-C, TAG, LDL-C, nitrate levels and PON1 activity in different studied groups

Parameters	Studied groups		
	Control	Colitis	Colitis AG treated
TC	59.79 ± 3.05	83.21 ± 2.34[a]**	71.55 ± 2.97[a]*,[b]*
HDL-C	39.61± 1.95	26.85 ± 2.78[a]**	36.49 ± 2.68[b]*
TAG	67.50 ± 5.56	82.44 ± 9.13	79.34 ± 6.77
LDL-C	8.851 ± 1.76	19.15 ± 2.04[a]*	13.20 ± 2.93
PONI	124.8 ± 6.35	83.50 ± 5.24[a]**	113.3 ± 4.75[b]*
Nitrate	16.47 ± 1.22	43.12 ± 3.69[a]**	15.65 ± 1.32[b]**

Units: TC, HDL-C, TAG, LDL-C mg/dl, PON1 U/l and nitrateμ mol/l; Data are represented as mean ± SEM of eight rats per group; [a] Significantly different from control group; [b] Significantly different from colitis group *P < 0.01; **P < 0.001.

Table 2: Colon tissue content of PCO, MDA and GSH content as well as SOD and CAT activity different studied groups

Parameters	Studied groups		
	Control	Colitis	Colitis AG treated
PCO	4.60 ± 0.69	12.77 ±1.07[a]**	6.81 ± 0.99[b]**
MDA	8.55 ± 1.07	30.47 ± 4.47 [a]**	12.07 ± 1.39[b]**
GSH	153.4 ± 9.79	36.15 ± 5.45 [a]**	132.7 ± 7.31[b]**
SOD	6.02 ± 0.67	1.03 ± 0.16 [a]**	5.18 ± 0.72[b]**
CAT	26.49 ± 2.58	10.30 ± 1.44 [a]**	24.67 ± 2.60[b]**

Units: PCO nmol/ mg protein, GSH μmol/mg protein, SOD U/ mg protein and CAT U/ mg protein
[a] Significantly different from control group; [b] Significantly different from colitis group; **P < 0.001

Discussion:

Oxidative damage to proteins increased PCO formation due to oxidation of sensitive amino acids; moreover lipids are peroxidized by ROS and liberate MDA. NO in cells is rapidly converted to nitrite, after conversion to nitrate can be determined as an indicator for NO production. Superoxide can be reacts with NO to produce peroxynitrite, which is considered a more powerful oxidant than superoxide [35] .Peroxynitrite diffused to the cells and nitrate variety of proteins molecules either at the aromatic rings or thiol groups, furthermore nitration of proteins is influence the protein functions [36].

In the present study, the serum nitrate level was significantly increased by inoculation of rats with AA. These results were in concurrence with a previous study reported that of the activity iNOS is unregulated in UC associated with excessive generation of NO [37]. Moreover another study demonstrated that the elevation nitrate level is indicator for the inflammation [38]. Intra-colonic administration of 4% AA was resulted increase of NO which converted to peroxynitrite mediates oxidative damage to biomolecules. This was demonstrated by significant reduction of colonic tissues content of GSH, SOD activity and CAT activity as antioxidants parameters. On another side PCO and MDA content as marker for oxidation of proteins and lipid were elevated. These findings are in accordance with previous study reported that oxidative stress is responsible for cellular damage associated with intestinal diseases[35, 39].

The treatment with AG ameliorates the levels of the nitrate as well as the above mentioned parameters compared with UC rats. These data are supported with several studies stated that, iNOS inhibit diamine oxidase activity in addition to ROS scavenging activity [40,41].

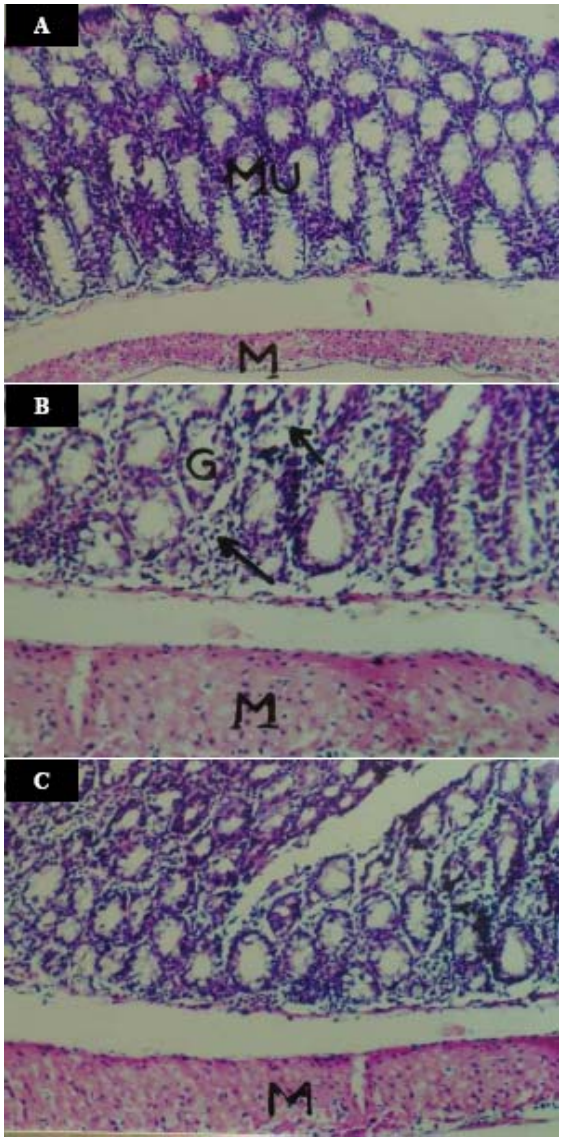

Figure 3: Colon section of rats in control group showing the normal histological appearance of the mucosal layer and *Lamina propria* (Mu) with underlying muscularis (M), H and E × 40 (A). Acetic acid-induced colitis in rats; colon section showing diffuse goblet cells formation in mucosal epithelium (G) with edema and diffuse inflammatory cells infiltration in *Lamina propria* (arrow) and hypertrophy in the muscularis (M) H and E ×64 (B). Treatment with AG before acetic acid inoculation showing goblet cells formation in the mucosal epithelium with no edema formation, H and E × 64 (C).

Moreover, supplementation of antioxidants protect against free radicals induced injury to the intestinal mucosa [42].With reference to these demonstrations the action of AG is mediated through inhibition of reduction of NO production and antioxidant activity.

PON1 has free cysteine residues that may be potentially target for the attack by these reactive species, may be resulting in altered enzymatic activity [43]. Serum PON1 activity was reduced in response to oxidative stress, furthermore its activity was improved in response to antioxidants supplementation [44]. Our investigation revealed that AA treated rats have lower serum PON1 activity than control rats, however PON1 is a HDL associated enzyme, the reduced serum PON1 activity is attributed to the decrease of HDL-C levels. These results are in agreement with a study suggested that decreased serum PON1 activity is due to oxidative stress associated with UC [45].Moreover the reduction of PON1 activity is due to the consumption of PON1 in the prevention of oxidative stress 46. Conversely antioxidants supplementations have been found to preserve PON1 activity[47]. The restoration of PON1 activity by AG supplementation before AA administration is due to its role in the protection against excessive RNS and ROS formation.

The colonic degeneration induced by AA administration was confirmed by histopathological examinations. However, platelets activating factor formation is responsible for the inflammatory processes associated with UC, these degenerations may be due to the ability of AA to increase the platelets activating factor formation. Furthermore, the reduction of HDL-C in UC rats may be also another cause of the increase of platelets activating factor because platelets activating factor acetyl-hydrolase is one of HDL-C associated enzymes [48]. The preservation of the intestinal physiology by AG as demonstrated by histopathological examination is probably the cause of HDL-C increase since the HDL proteins are produced in the intestine.

Conclusion:

Increase of NO derived from iNOS plays an important role in the pathogenesis of UC.This effect is mediated through increase of production both ROS and RNS resulted in decrease of colonic tissues GSH contents as well as attenuation of PON1, SOD and CAT activities. Moreover, increase the tissues both lipids and proteins oxidation. Restoration of NO level by AG is responsible for the preservation of PON1, SOD, CAT activities as well as attenuation of proteins and lipids oxidation. These results indicated that AG treatment reduce of inflammatory reactions associated with UC as confirmed with histopathological investigations.

Acknowledgement:

The author would like to thank *Kayyali* Research Chair for Pharmaceutical Industries, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia for the assistance in completion of this work. Also, acknowledging Dr. Adel Bekairy, Professor of Histopathology, Cairo University, Egypt for his great help in this research in the histopathological examination of colon specimens.

References:

[1] D. P. Rosanna, M. Emanuela, S.A. P. Nimesh, G. Tiziana, M. Carmelo, T. Christoph, D. S. Angelina, and C. Salvatore. Beneficial effects of GW274150 treatment on the development of experimental colitis induced by dinitrobenzene sulfonic acid, Eur. J. Pharmacol 507: 281– 289 (2005).

[2] C.F. Krieglstein, W.H. Cerwinka, F.S. Laroux , J.W. Salter, J.M. Russell, G. Schuermann, M.B. Grisham, C.R. Ross, and D.N. Granger. Regulation of murine intestinal inflammation by reactive metabolites of oxygen and nitrogen, J. Exp. Med. 194: 1207– 1218 (2001).

[3] I. Singer, D.W. Kawka, S. Scott, J.R. Weidner, R.A.Mumford, T.E., Riehl, and W.F., Stenson. Expression of inducible nitric oxide synthase and nitrotyrosine in colonic epithelium in inflammatory bowel disease, Gastroenterology 111: 871–885(1996).

[4] H. Kimura, R. Hokari, S. Miura, T. Shigematsu, M. Hirokawa, Y. Akiba, I. Kurose, H. Higuchi, H. Fujimori, Y. Tsuzuki, H. Serizawa, and H. Ishii. Increased expression of an inducible isoform of nitric oxide synthase and the formation of peroxynitrite in colonic mucosa of patients with active ulcerative colitis, Gut 42: 180–187 (1998).

[5] M. Harma, M. Harma, and O. Erel. Increased oxidative stress in patients with hydatidiform mole, Swiss Med Wkly 133: 563-566 (2003).

[6] G.D. Buffinton, and W.F. Doe. Depleted mucosal antioxidant defenses in inflammatory bowel disease, Free Radic. Biol. Med. 19 (6): 911-918 (1995).

[7] Z. Serdar, K. Aslan, M. Dirican, E. Sarandol, D. Yesilbursa, A. Serdar. Lipid and protein oxidation and antioxidant status in patients with angiographically proven coronary artery disease, Clin Biochem 39: 794-803 (2006).

[8] K. Gan, A. Smolen, H. Eckerson, and B. La Du Purification of human serum paraoxonase / arylesterase. Evidence for one esterase catalyzing both activities, Drug. Metab. Dispos. 19: 100 (1991):

[9] O. Rozenberg, M. Rosenblat, R. Coleman, D.M. Shih, and M. Aviram. Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice, Free Radic Biol Med 34: 774-784 (2003).

[10] G. Baskol, H. Demir, and M. Baskol. Assessment of paraoxonase 1 activity and malondialdehyde levels in patients with rheumatoid arthritis, Clin Biochem 38: 951-955 (2005).

[11] G. Basko, S. Karakucuk, and A. Oner. Serum paraoxonase 1 activity and lipid peroxidation levels in patients with age related macular degeneration, Ophthalmologica 220: 12-16 (2006).

[12] M. Baskol, G. Baskol, K. Deniz, O. Ozbakýr, and M. Yucesoy. A new marker for lipid peroxidation: serum paraoxonase activity in non-alcoholic steatohepatitis, Turk J Gastroenterol 16: 119-123 (2005).

[13] S. Karakucuk, G. Baskol, A. Oner, M. Baskol, E. Mirza, and M. Ustdal. Serum paraoxonase activity is decreased in the active stage of Behcet.s disease, Br J Ophthalmol 88: 1256-1258 (2004).

[14] G. Dijkstra, H.M. Moshage, A. Jager-Krikken, A.T Tiebosch, J.H. Kleibeuker, P.L. Jansen, and H. Van Goor. Expression of nitric oxide synthases and formation of nitrotyrosine and reactive oxygen species in inflammatory bowel disease, J. Pathol. 186: 416–421 (1998).

[15] Y.Yoshida, A. Iwai, K. Itoh, M.Tanaka, S.Kato, R. Hokari, T. Miyahara, H. Koyama, S.Miura, M., and Kobayashi. Role of

inducible nitric oxide synthase in dextran sulfate sodium-induced colitis, Aliment. Pharmacol. Ther. 14: 26–32 (2000).

[16] T. Saulis, M.E. Cooper, S. Sastra, V. Thallas, S. Panagiotopoulos, O.J. Bjerrum, and G. Jerums. Relative contributions of advanced glycation and nitric oxide synthase inhibition to aminoguanidine mediated renoprotection in diabetic rats, Diabetologia 40: 1141-1151(1997).

[17] K. Kedzora-Karnotowska, and M.Luciak. Effect of aminoguanidine on lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney, Biochem Mol Biol Int 46 (3): 577-583 (1998).

[18] B.R. MacPherson and C.J. Pfeiffer. Experimental production of diffuse colitis in rats, Digestion 17: 135–150 (1978).

[19] T. Saulis, M.E.Cooper, S. Sastra, V. Thallas, S. Panagiotopoulos, O.J. Bjerrum, and G. Jerums. Relative contributions of advanced glycation and nitric oxide synthase inhibition to aminoguanidine mediated renoprotection in diabetic rats, Diabetologia **40**: 1141-1151(1997).

[20] A. Cetinkaya, E. Bulbuloglu, E.B. Kurutas, H. Ciralik, B. Kantarceken, and M.A. Buyukbese. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats,Tohoku J. Exp. Med. 206(2):131-139 (2005).

[21] J.L. Wallace, C.M. Keenan, D. Gale, and T.S. Shoupe. Exacerbation of experimental colitis by nonsteroidal anti-inflammatory drugs is not related to elevated leukotriene B4 synthesis, Gastroenterology 102: 18–27 (1992).

[22] P. Roeschlau, E. Bernt, and W. Gruber. Enzymatic determination of total cholesterol in serum, Z Klin Chem Klin Biochem 12: 226 (1974).

[23] M. Burstein, H. Scholnick, and R. Morfin. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions, J Lipid Res 11: 583–95 (1970).

[24] G. Bucolo and H. David. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19: 476–82 (1973).

[25] W. Friedewald, R. Levy and D. Fredrickson. Estimation of the concentration of low–density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin Chem 18: 499–502 (1972).

[26] H.W. Eckerson, C.M. Wyte,and B.N. La Du. The human serum paraoxonase/arylesterase polymorphism, Am J Hum Genet 35: 1126-38 (1983).

[27] P.N. Bories and C. Bories. Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase, Clin. Chem. 41(6): 904-907(1995).

[28] J.M. Fagan, B.G. Sleczka, and I. Sohar. Quantitation of oxidative damage to tissue proteins, Int J Biochem Cell Biol 31:751-757(1999).

[29] S.L. Marklund. Product of extracellular-superoxide dismutase catalysis, FEBS Lett. 184 (2): 237-239 (1985).

[30] N. Siveski – Iliskovic and N. Kaul. Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats, Circulation 89 (6): 2829-2835 (1994).

[31] M. Ellman (1959). A spectrophotometric method for determination of reduced glutathione in tissues, Analyt. Biochem.74: 214-226.

[32] M. Mihara and M. Uchiyama. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test, Anal. Biochem. 86(1): 271-278 (1978).

[33] O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall. Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193(1): 265-275 (1951).

[34] J.D. Banchrof, A. Steven, and D Turner.Theory and practice of histopathological techniques, Fourth Ed,Churchil Livingstone, New tork, London , San Francisco, Tokyo, 1996 pp.109.

[35] L. Jing-Li, D. Jun, F. Ling-Ling, L. Xiao-Yan, G. Luo, and G.Ying-Bin. Effects of quercetin on hyper-proliferation of gastric mucosal cells in rats treated with chronic oral ethanol through the reactive oxygen species-nitric oxide pathway,World J Gastroenterol 14(20): 3242-3248 (2008).

[36] R. Radi, G. Peluffo, M.N. Alvarez, M Naviliat, and A. Cayota. Unraveling peroxynitrite formation in biological systems, Free Radic Biol Med 30: 463-488 (2001).

[37] A. Perner, L. Andresen, M. Normark, B.Fischer-Hansen, S. Sorensen and J. Eugen-Olsen. Expression of nitric oxide synthases and effects of L-arginine and LNMM on nitric oxide production and fluid transport in collagenous colitis, Gut 49(3): 387-394 (2001).

[38] J.O. Lundberg, M. Herulf, M. Olesen, J. Bohr, C. Tysk, N.P. Wiklund, E. Morcos, P.M. Hellstrom, E. Weitzberg and G. Jarnerot. Increased nitric oxide production in collagenous and lymphocytic colitis, Eur. J. Clin. Invest. 27(10): 869-871. (1997).

[39] J. Tanaka and Y. Yuda. Lipid peroxidation in gastric mucosal lesions induced by indomethacin in rats, Biol. Pharm. Bull. 19: 716-720 (1996).

[40] R.G. Tilton, K. Chang, and K.S. Kasan. Prevention of diabetic vascular dysfunction by

guanidines. Inhibition of NOS versus advanced glycation end product formation, Diabetes, 42: 221-232 (1993).

[41] C. Szabo, G. Ferrer-sneta, B. G.J. Zingarelli, Southan, A.L. Salzman and R. Radi . Mercapto ethyl guanidine and guanidine inhibitors of nitric oxide synthase react with peroxynitrite and protect against peroxynitrite-induced oxidative damage, J. Biol. Chem. 272: 9030- 9036 (1997).

[42] N. Sugimoto, N.Yoshida, T. Yoshikawa, Y. Nakamuara, H. Ichikawa, Y. Naito, and M. Kondo. Effect of Vitamin E on aspirin-induced mucosal injury in rats,Dig. Dis. Sci., 45: 599-605 (2000).

[43] M.I. Mackness**,** B**.** Mackness, P.N. Durrington, P.W. Connelly, and R.A. Hegele. Paraoxonase: biochemistry, genetics and relationship to plasma lipoproteins, Curr Opin Lipidol **7:** 69-76 (1996).

[44] R. Frank, M. Zwart, J. Kastelein and H. Voorbij. PON_2 gene variants are associated with clinical manifestations of cardiovascular disease in familial hypocholesterolemic patients, Atheroscl, 154: 641- 649 (2001).

[45] G. Baskol, M. Baskol, A. Yurci, O. Ozbakir, and M.Yucesoy .Serum paraoxonase-1 activity and malondialdehyde levels in patients with ulcerative colitis, Cell Biochem Funct.24: 283– 286 (2006).

[46] M. Aviram., M. Rosenblat, and S. Billecke .Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants, Free Radical Biol Med. 26: 892– 904 (1999).

[47] P.N. Durrington, B. Mackness and M.I. Mackness. The hunt for nutritional and pharmacological modulators of paraoxonase, Arterioscler Thromb Vasc Biol 22:1248-1250 (2002).

[48] K. Ken, H. Ayako, S.Noriko, I. Keizo, and S. Morio. Plasma platelet activating factor-acetylhydrolase (PAF-AH), Progr Lipid Res 42 : 93–114(2003)