ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Vitamin E is Effective in Treatment of Ulcerative Colitis Induced by Iodoacetamide in Rats

Mohamed G.Ewees[1], Basim A. Shehata[2], Ali A. Aboseif[3], Hekma A. Abd El-Latif[4]

1. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt
2. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University, Egypt
3. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt
4. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Umm al Qura University, Makah, KSA

Manuscript Info

Manuscript History:

Received: 15 October 2014
Final Accepted: 26 November 2014
Published Online: December 2014

Key words:
Ulcerative colitis – Vitamin E - Iodoacetamide

Corresponding Author

Mohamed G.Ewees

Abstract

Ulcerative colitis, is a life-threatening chronic inflammatory disease which affects the gastrointestinal tract especially colon and rectum**.** The present investigation aims to elucidate the possible protective effective of vitamin E on ulcerative colitis induced by administration of iodoacetamide to adult male albino rats. Animals were divided into (4) groups, each of 8 rats. The first group was kept as normal control group. The other three groups were once administrated 0.1 ml of 3% iodoacetamide rectally then treated with saline in ulcerative colitis control, sulfasalazine in standard and vitamin E in treatment group on a daily basis for (7) consecutive days starting from the day of induction. Animals were sacrificed (24) h after the lost dose and tissue samples were collected. Administration of iodoacetamide induced UC in rats evidenced by significant increase in colon ulcer score, colon weight/length ratio, colon myeloperoxidase (MPO) activity, colon total nitrate/nitrite (NOx) production and colon malondialdehyde (MDA) content, In addition, significant decrease in colon superoxide dismutase (SOD) activity and colon glutathione (GSH) content. Treatment with sulfasalazine or vitamin E improved UC as evidenced by significant decrease in colon ulcer score, colon weight/length ratio, colon MPO activity, colon NOx production and colon MDA content, In addition, significant increase in colon CAT activity and colon GSH content. It seems that vitamin E is protective against UC in rats and is promising for further chemical trials.

Introduction

Ulcerative colitis (UC), is a common, chronic inflammatory disease which affects the gastrointestinal tract especially colon and rectum **(Viscido et al., 2005)**. Usually, it is mildly active, but it can be life-threatening during severe attacks because of colonic and systemic complications. In addition, patients with long-term ulcerative colitis are at an increased risk of colorectal cancer **(Ekbom et al., 1990)**.

The exact cause of UC remains unknown, but possible etiological factors, including genetic, immunologic and environmental factors may be involved **(Jewell and Patel, 1985)**. In addition, oxidative stress has been involved in the pathogenesis of ulcerative colitis in experimental animals and in humans **(Keshavarzian et al., 1990; Kitahora et al., 1998)**.

It is known that the balance between the antioxidants in the intestinal mucosa is seriously impaired in UC patients compared to normal mucosa**,** where intestinal inflammation is associated with excessive production of reactive oxygen and nitrogen metabolites **(Kruidenier and Verspaget, 2002).**

Treatment of UC aimed to remission of symptoms and mucosal inflammation. Most of the current therapies for UC involve treatment with corticosteroids and 5-aminosalicylic acid **(Podolsky, 1991; Strober et al., 1998)**. Unfortunately, these drugs have serious side effects, which will limit their use **(Michel, 1999)**. On the other hand, antioxidant therapy has shown beneficial effects in several experimental models of rat colitis **(Son, 1998)**

Vitamin E is the most important lipophilic antioxidant. It is considered as the 'standard antioxidant' due to its role in the prevention of cellular injury associated with oxidative stress **(Burton, 1994; Brigelius-Flohe and Traber, 1999)**. In this investigation, the antioxidant effect of vitamin E was tested against sulfasalazine in (IA) induced ulcerative colitis in rats.

2. Material And Method:

2.1 Animals:

Animals weighing (180-200 g) were used in this study. Animals were obtained from the Modern Veterinary Office for Laboratory Animals, Cairo, Egypt. The rats were kept under standard conditions of temperature (25°C ± 0.5) and relative humidity (55 ± 1) with 12-light/12-dark cycle for one week for adaptation before being subjected to laboratory experiments and were allowed free access to standard forage and drinking water ad libitum. Experimental protocol was designed according to the regulation of ethical committee Faculty of Pharmacy Beni-Sweif University.

2.2 Drugs and chemicals:

Sulfasalazine was provided as a gift from Acdima International Company (Egypt), whereas vitamin E (DL-α tocopherol acetate) and iodoacetamide were purchased from Sigma-Aldrich (USA).

2.3 Induction of experimental colitis in rats:

Ulcerative colitis (UC) was induced in rats by intra-colonic instillation of 0.1 ml 3% iodoacetamide dissolved in 1% carboxy methyl cellulose (CMC) using medical grade canal for enteral feeding (diameter 2 mm) where the tip of canal was advanced to 8 cm proximal to the anus verge after which fluid was withdrawn. Animals were allowed to hang in air by holding their tails for 1-2 min to prevent spillage of solution from rectum. Rats were left for 7 days to induce colitis **(Shibolet et al. 2002).**

2.4 Experimental design:

Thirty two adult male albino rats (180–200 g) were randomly assigned to four groups, each of (8) rats. The first group was kept as normal control group. The other three groups were once administrated 0.1 ml of 3% (IA) rectally together with either vehicle ($10ml.kg^{-1}$/day .p.o.; ulcer control group), sulfasalazine ($500mg.kg^{-1}$/day p.o.; **Mustafa, 2006**) or vitamin E ($30mg.kg^{-1}$/day p.o.; **Tahan et al., 2011**). All treatments were given after IA and continued for 7 consecutive days. Drugs and (IA) were suspended in 1% CMC/tween 80 solution.

2.5 Assessment of colitis:

2.5.1 Colon weight/length Ratio

At the end of the experiment, animals were sacrificed, the abdominal cavities opened, The distal 9 cm portion of the colon was removed and cut longitudinally, slightly cleaned in ice cold saline to remove fecal residues then dried with filter papers and weighed. Colon was subjected to microscopical and histopathological examination assesses the degree of colon edema which reflected the severity of colitis **(Hossam et al., 2009)**.

2.5.2 Histopathological study:

Samples were fixed in 10% formal saline for histological examination. Samples were embedded in paraffin, and sections were stained with haematoxylin and eosin for histological evaluation of colonic damage by light microscopy.

2.5.3: Macroscopic scoring:

Macroscopic appearance of the colonic mucosa was scored according to a scale ranging from 0 to 4, where (0) means No macroscopic changes, (1) means mucosal erythema only, (2) means mild mucosal oedema, slight bleeding or small erosions, (3) means moderate oedema, bleeding ulcers or erosions and (4) means severe ulceration, erosions, oedema and tissue necrosis **(Millar et al, 1996).**

2.6 Preparation of tissue homogenate:

A portion of the colon was homogenized with 10 volumes of isotonic ice-cooled normal saline using a homogenizer (Ultra-Turrax T_{25}, IKA Labortechnik, Germany) to prepare 10% homogenate. The homogenate was centrifuged at 4000 xg for 15 minutes. The obtained supernatant was used for the measurement of MPO activity, NOx, MDA and antioxidants (SOD, CAT and GSH).

2.7 Determination of myeloperoxidase activity MPO:

Myeloperoxidase (MPO) activity served as quantitative index of neutrophil infiltration and inflammation in several tissues, including the intestine **(Bradley et al., 1982).** Myeloperoxidase (MPO) activity was determined in colon homogenate spectrophotometrically according to the method of **Krawisz et al. (1984)**.

2.8 Determination of total nitrate/nitrite (NOx) ratio:

Ulcerative colitis was found to be accompanied with an increase of NO production by iNOS **(Zhou and Yu 2007; Martı´n et al. 2007**). The levels of NO and iNOS activities in the colon tissues were, measured spectrophotometrically at 540 nm. according to the method described by **Miranda et al. (2001).**

2.9 Determination of Lipid peroxidation:

Lipid peroxidation products were estimated by the determination of the level of thiobarbituric acid reactive species (TBARS) that were measured as malondialdehyde (MDA) in the colon homogenates spectrophotometrically according to **Uchiyama and Mihara (1978)**.

2.10 Determination of reduced glutathione:

Glutathione was measured in Colon homogenate a according to the method described by **Sedlak and Lindsay (1968)**. The principle of the method depends on the reduction of 5,5`-Dithiobis-(2-nitrobenzoic acid) (DTNB) by the sulfhydryl group of GSH. The formed product was measured calorimetrically at 412 nm. Results were expressed as µmol/g tissue.

2.11 Determination of superoxide dismutase (SOD) activity

Determination of SOD activity in the colon homogenate supernatant was based on inhibition of pyrogallol auto-oxidation as described by **Marklund (1985).** Samples were measured spectrophotometrically at absorbance 420 nm at 1 min interval for 3 min. Results were expressed as U/g tissue.

2.12 Determination of Catalase (CAT) activity:

Catalase activity was measured in the Colonic tissues according to **Clairborne (1985)**, The principle of this method depending on the decrease in catalase activity due to the decomposition of hydrogen peroxide at 240 nm. Enzyme activity was expressed as U/g tissue.

2.13 Statistical analysis:

All data are expressed as mean±standard error (S.E.) of 8 rats per experimental group. Statistical analysis was performed using one-way ANOVA followed by Student-Newman-keuls multiple comparisons test by the aid of Graph bad prism and Graph pad instant computer software, San Diego, USA. $P<0.05$ was considered significant.

Results:

Assessment of colitis:

On histological examination of rat colon from the normal group, the histological features were typically of a normal structure (Fig, 1A)

Intra-colonic administration of 0.1 ml of iodoacetamide (3%) caused extensive macroscopic damage of the colon and the colonic mucosa appeared hemorrhagic and ulcerated and characterized by loss of surface epithelium and crypts, submucosal oedema with inflammatory reaction in lamina propria and crypt abscess (Fig, 1B). The inflammatory process was associated also with increase of both; colonic weight/length ratio and colonic ulceration score (Fig; 2).

Treatment with sulfasalazine 500mg.kg^{-1} or vitamin E 30mg.kg^{-1} attenuated the extent and severity of the histological signs of tissue damage in colon tissues (Fig; 1C,1D) with significant decrease in colon ulcer score and colon weight length ratio (Fig; 2).

Colon MPO activity:

The MPO activity of the colon tissue significantly increased in the IA group as compared with the normal group (Fig, 3), while treatment with sulfasalazine or vitamin E significantly decreased colon MPO activity (Fig, 3)

Total nitrate/nitrite (NOx) ratio:

Intra-colonic administration of iodocetamide significantly increased colon NOx production as compared with normal control (Fig, 3). On the other hand treatment with sulfasalazine or vitamin E significantly decreased colon NOx production (Fig, 3)

Colon MDA content:

Colon MDA content significantly increased in iodoacetamide group as compared to normal control (Fig, 4) while significant decrease in colon MDA content occurred after treatment of rats with sulfasalazine or vitamin E (Fig, 4)

Colon content of reduced glutathione:

Compared to normal control group, colon GSH content significantly decreased in iodoacetamide group (Fig, 4). After treatment with sulfasalazine or vitamin E a significant increase in colon GSH content was occurred (Fig, 4)

Colon superoxide dismutase (SOD) activity

The colon SOD activity significantly decreased after intra-colonic administration of iodoacetamide as compared with normal control group (Fig, 5). Treatment with sulfasalazine or vitamin E were able to increase the colonic tissue SOD activity but not in a significant manner (Fig, 5)

Colon Catalase (CAT) activity:

Intra-colonic administration of iodoacetamide significantly decreased the colon CAT activity as compared to normal control group (Fig, 5), meanwhile treatment with sulfasalazine or vitamin E significantly increased the colonic tissue CAT activity (Fig, 5)

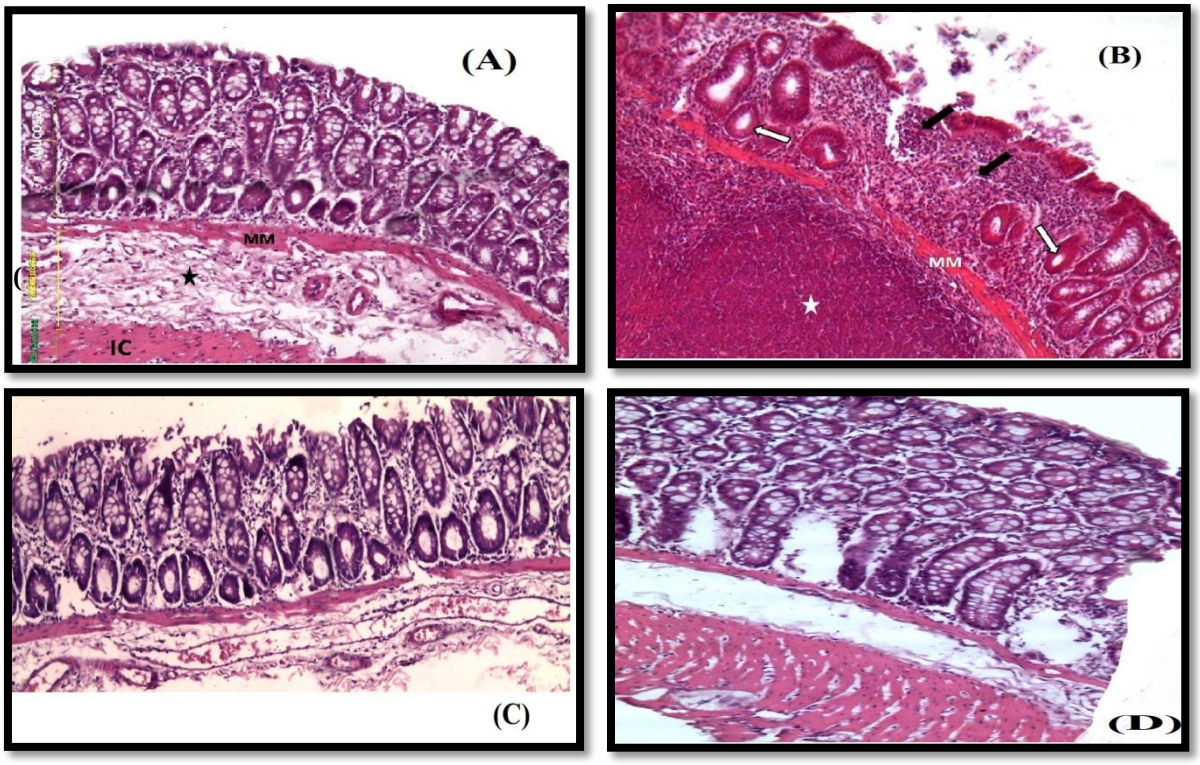
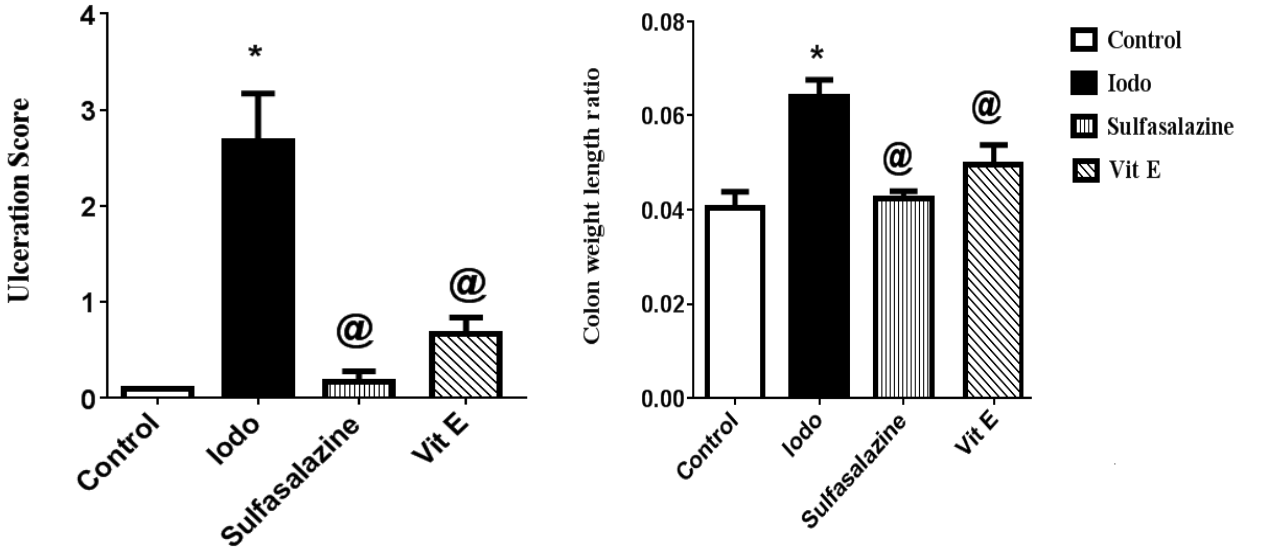


Fig 1): (A) Histological colonic mucosal sections of normal rat showing normal mucosa with intact epithelial surface. (B) Iodoacetamide induced colitis showed Loss of crypts with diffuse inflammatory reaction of both lamina propria and submucosa, Deformed crypts are observed and muscularis mucosa (MM) is disrupted. (C) Sulfasalazine drug, (D) vitamin E and both attenuated the extent and severity of cell damage.



(Fig 2): Effect of one week daily oral treatment with vitamin E on colon ulceration score and colon weight length ratio as compared to sulfasalazine on ulcerative colitis induced by iodoacetamide in rats.
Each bar represents the mean of 8 experiments ± SEM
- Statistical analysis was performed using one-way ANOVA followed by Student-Newman-keuls multiple comparisons test.
* Significantly different from sham control value at $p < 0.05$
@ Significantly different from ulcer control value at $P < 0.05$

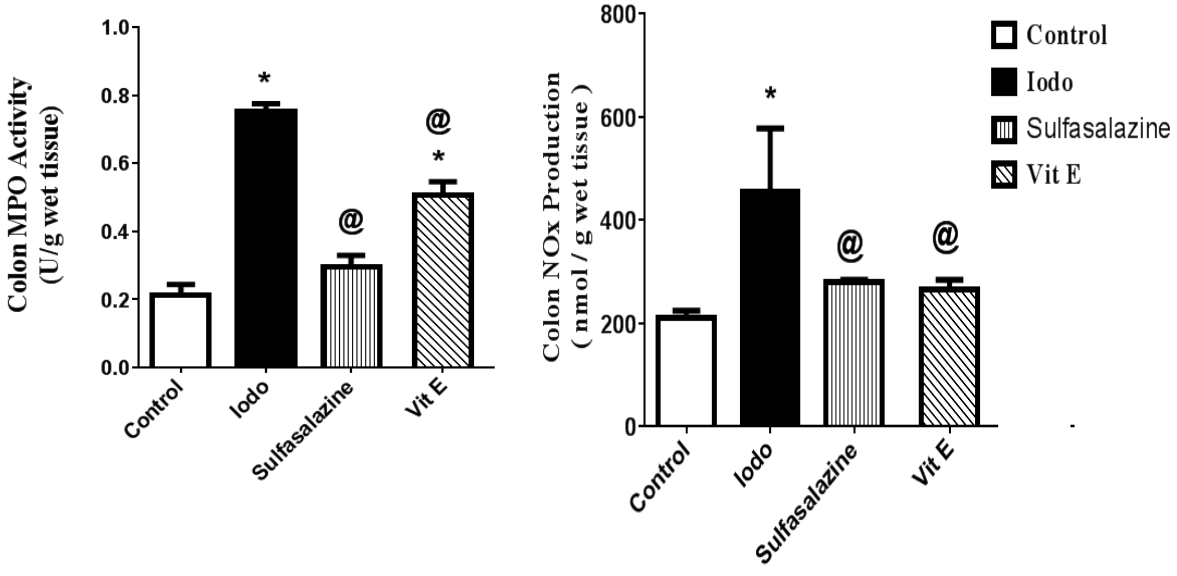


Fig (3): Effect of one week daily oral treatment with vitamin E on colon MPO activity and colon NOx production as compared to sulfasalazine on ulcerative colitis induced by iodoacetamide in rats
Each value represents the mean of 8 experiments ± SEM
- Statistical analysis was performed using one-way ANOVA followed by Student-Newman-keuls multiple comparisons test.
* Significantly different from sham control value at $p < 0.05$
@ Significantly different from ulcer control value at $P < 0.05$

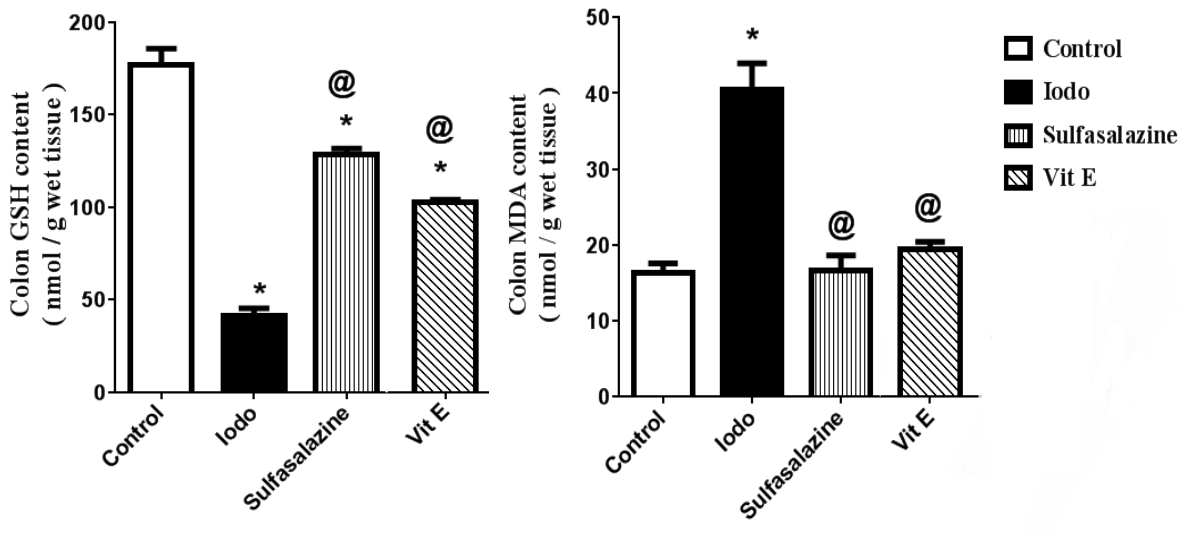


Fig (4): Effect of one week daily oral treatment with vitamin E on colon GSH content and colon MDA content as compared to sulfasalazine on ulcerative colitis induced by iodoacetamide in rats
Each value represents the mean of 8 experiments ± SEM
- Statistical analysis was performed using one-way ANOVA followed by Student-Newman-keuls multiple comparisons test.
* Significantly different from sham control value at $p < 0.05$
@ Significantly different from ulcer control value at $P < 0.05$

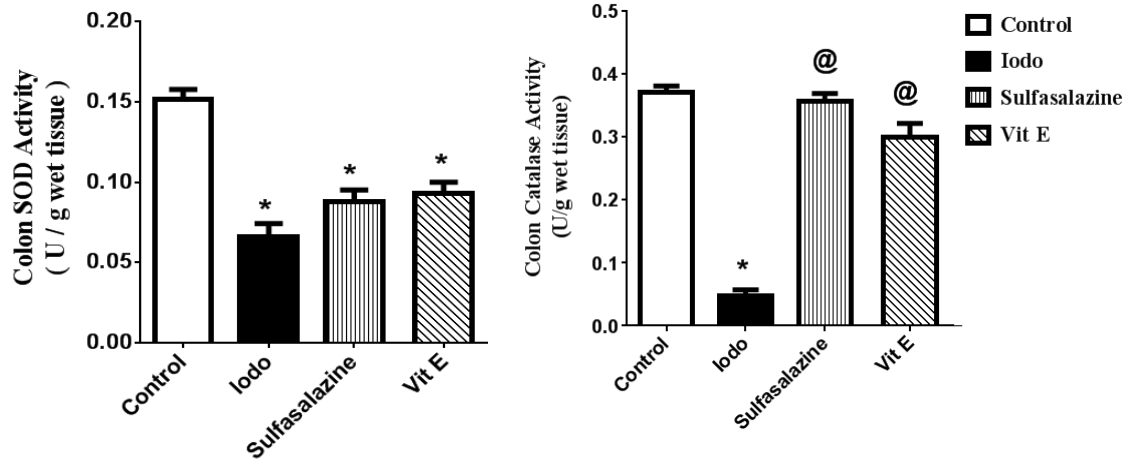


Fig (5): Effect of one week daily oral treatment with vitamin E on colon SOD activity and colon CAT activity as compared to sulfasalazine on ulcerative colitis induced by iodoacetamide in rats

Each value represents the mean of 6-8 experiments ± SEM
- Statistical analysis was performed using one-way ANOVA followed by Student-Newman-keuls multiple comparisons test.
* Significantly different from sham control value at $p < 0.05$
@ Significantly different from ulcer control value at $P < 0.05$

Discussion:

It is important to know that treatment of UC is difficult because of its complex etiology **(Medhi et al. 2008).** The exact cause of UC remains unknown, but possible etiological factors have been involved, including genetic, immunologic and environmental **(Jewell and Patel 1985)**.

Many experimental models have been developed for UC in a trial to get the exact events that illustrate the disease **(Jurjus et al., 2004; Hajj et al., 2008; Arafa et al., 2009)**. The most common chemicals used to induce models causing acute destruction of the intestinal barrier are trinitrobenzene sulphonic acid (TNBS) **(Neurath et al., 1995)**, dextran sodium sulphate (DSS) **(Okayasu et al., 1990)** and oxazolone **(Boirivant et al., 1998)**. These models have different advantages and disadvantages that have been greatly reviewed by Hoffmann et al. **(2002).**

In this study, the mucosal inflammation was induced by the sulfhydryl blocker, iodoacetamide (IA). It is an alkylating agent which caused mucosal injury by blocking sulfhydryl groups that are very important for the vitality of the intestine **(Satoh et al., 1997)**.

Using of sulfhydryl blockers as chemical inducers of inflammation was rarely applied. However, it was proved that IA increased vascular permeability, caused massive mucosal edema, erosion and ulcers **(Rachmilewitz et al., 1997; Satoh et al., 1997; Tolstanova et al., 2012)**, Therefore, IA induced colitis model was used to reflect the histological characteristics of the diseases and sharing many characteristics with human colitis.

In the present investigation, intra-colonic administration of IA (3%) to rats caused severe colon ulceration evidenced macroscopically by extensive macroscopic damage of the colon and significant increase in colon ulceration score. histologically; where the colonic mucosa appeared hemorrhagic, ulcerated and characterized by loss of surface epithelium, crypts and sub-mucosal oedema with inflammatory reaction in lamina propria and Crypt abscess.in addition, IA significantly increased colon weight/length ratio and significantly increased colon MPO activity and colon NOx production.

This model is based on the fact that endogenous sulfhydryl (SH) compounds, such as glutathione, play a crucial role in the protection of gastric mucosa (**Satok and Szabo, 1990)**. The decrease in the anti-oxidant defense mechanisms leads to increase in lipid peroxidation causing a destruction and damage to cell membranes. In this study, IA significantly increased colon MDA content and significantly decreased in colon GSH content, colon SOD activity and colon CAT activity.

Sulfasalazine drug is considered as standard drug for management of ulcerative colitis. It is composed of 5-aminosalicylic acid (5-ASA) with anti-inflammatory activity linked to sulfapyridine with antibacterial activity through a diazo bond, this bond is readily cleaved by bacterial azoreductases in the colon **(Peppercorn and Goldman 1972)**.

The anti-inflammatory effect of sulfasalazine was already confirmed in this study according the obtained results by improving the inflammatory markers through significant reducing of colonic damage score and also significant attenuation of the extent and severity of the histological signs of cell damage. There were no inflammatory cells in the lamina propria and the epithelium remained intact so it is fined that sulfasalazine significantly decreased colon weight/egnth ratio. This results is in harmony with the work of other investigators who reported similar findings (**Hagar et al., 2007: Ballester et al., 2005).**

The anti-inflammatory effect of sulfasalazine was proved also by reducing neutrophil infiltration as evidenced by decreased MPO activity and thus decreasing mucosal ulceration **(Cestari, Bastos and Di Stasi, 2011)**. The anti-inflammatory properties of sulfasalazine are related to the (5-ASA) moiety, because it interferes with arachidonic acid metabolism **(Peppercorn, 1984)**. In addition, it has a role in inhibition of chemo-taxis of inflammatory cells, inhibition of expression of cytokine such as IL-1, IL6, tumor necrosis factor α (TNF-α), in addition to inhibition of lymphocyte proliferation and activation **(Plosker and Croom, 2005)**.

The anti-oxidant properties of sulfasalazine was proved in this study through increasing colon GSH content and colon catalase activity. These findings are in agreement with the results of other investigators who reported similar increase in GSH content **(Witaicenis et al., 2012)**, similar increase in colon SOD activity **(Wadie et al., 2012)** and similar increase in CAT activity **(Das and Kanodia, 2012**), on the other hand, sulfasalazine significantly decreased colon MDA content similarly to **Dirlik et al. (2009).**

Finally, we can say that the activity of sulfasalazine in treatment of ulcerative colitis not only related to its anti-inflammatory activity but also related to its antioxidant and free radical scavenger properties **(Miles and Grisham,**

1994). The radical scavenger effect of 5-ASA has also been suggested from observations on its ability to prevent xanthine oxidase induced de polymerization of hyaluronic acid **(Carlin, 1985).**

Ulcerative colitis is characterized by intense oxidative stress which means an imbalance between the oxidative free radicle molecules and the defense provided by antioxidative mechanisms like glutathione, SOD enzyme, CAT enzyme **(Kruidenier et al., 2003)**. Therefore it is commonly accepted that oxidative stress is an important determinant of UC pathophysiology **(Kruidenier and Verspaget, 2002; Yamada and Grisham, 1991)**.

Therefore it is thought that antioxidants have a role in treatment of ulcerative colitis especially after proving serious side effects of drugs which already used to treat UC as sulfasalazine which make their use is limited **(Michel, 1999)**. This study is aimed to reveal the role of vitamin E in treatment of ulcerative colitis.

Vitamin E is considered as the 'standard antioxidant' due to its role in the prevention of cellular injury associated with oxidative stress **(Burton, 1994; Brigelius-Flohe and Traber, 1999)**. In this study, vitamin E significantly increased colon GSH content and colon CAT activity. It is also increased colon SOD activity but not in significant manner.

The anti-oxidative property of vitamin E was already recognized in the early 1930s. Since then, it has been classified as the major lipid-soluble antioxidant which protects lipids and membranes from oxidative damage in vitro and in vivo **(Tappel and Zalkin, 1960; Burton and Ingold, 1981; Burton et al., 1982)**.

The antioxidant property of vitamin E could be explained through its radical chain-breaking antioxidant properties **(Bjelakovic et al., 2007)** where Vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins, therefore it was also observed a significant decrease in colon MDA content by treatment with vitamin E.

In this study, vitamin E showed potent anti-inflammatory effect through improving the histological character of colon membrane, significant decreasing in colon ulceration score. Vitamin E also showed a significant decrease in colon weight/length ratio which consider a marker for degree of inflammation **(Rachmilewitz et al., 1997)**. In addition, Vitamin E significantly decreased colon MPO activity and colon NOx production.

The anti-inflammatory effect of vitamin E is related to inhibition of neutrophil function or cytokine production in colon mucosa **(Morris et al., 1989; Isozaki, 2006; Mirbagheri, 2008),** where Vitamin E inhibits the activity of the enzyme protein kinase C (PKC) which is associated with inflammation **(Munoz, 1989),** or through inhibition the release of IL-1b from lipopolysaccharide activated macrophages and adhesion of monocytes to endothelial cells **(Brigelius-Flohe´ et al., 2002)**. Vitamin E inhibits key events in inflammatory signaling, such as platelet aggregation, where oxidized alpha-tocopherol is also a powerful anticoagulant. In addition, vitamin E compounds reduce the production of inflammatory compounds such as prostaglandins **(Feranchak, et al., 1999).**

Conclusion:

In conclusion we suggest that vitamin E may be a promising agent for the prophylaxis of UC in comparison to sulfasalazine and could be used as a functional food in dietary supplements. Further sufficient preclinical and clinical studies should be conducted to clarify this fact.

References:

Arafa, H.M., Hemeida, R.A., El-Bahrawy, A.I. and Hamada, F.M. (2009): Prophylactic role of curcumin in dextran sulfate sodium (DSS)-induced ulcerative colitis murine model. Food Chem. Toxicol, 47: 1311-1317.

Ballester, I., González, R., Nieto, A., Zarzuelo, A., and de Medina, F. S. (2005): Monochloramine induces acute and protracted colitis in the rat: response to pharmacological treatment. Life sciences, 76(25): 2965–2980.

Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.G. and Gluud, C. (2007): Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. J. Am. Med. Assoc., 297(8): 842-857.

Boirivant, M., Fuss, I. J., Chu, A. and Strober,W. (1998): Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J. Exp. Med, 188: 1929-1939.

Bradley, P.D., Friebal, D.A. and Christensen, R.D. (1982): Measurements of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol, 78: 206–209.

Brigelius-Flohe, R. and Traber, M.G. (1999): Vitamin E: function and metabolism. FASEB J, 13: 1145–1155.

Brigelius-Flohe´, R., Kelly, F.J., Salonen, J., Neuzil, J., Zingg, J.M. and Azzi, A. (2002): The European perspective on vitamin E: current knowledge and future research. American Journal of Clinical Nutrition, 76: 703–716.

Burton, G.W. and Ingold, K.U. (1981): Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chainbreaking phenolic antioxidants in vitro. Journal of the American Chemical Society, 103: 6472–6477.

Burton, G.W., Joyce, A. and Ingold, K.U. (1982): First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. Lancet, 2: 327.

Burton, G.W. (1994): Vitamin E: molecular and biological function. Proc Nutr Soc., 53: 251–262.

Carlin, G., Djursäter, R., Goldschmidt, T., Smedegård, G. and Arfors, K.E. (1985): Degradation of hyaluronic acid and collagen by oxygen derived radicals. Inflammation. Basic Mechanisms, Tissue Injuring Principles, and Clinical Models. Eds P Venge & A Lindbom. Stockholm: Almqvist & Wiksell Int, pp 297-301.

Cestari, S. H., Bastos, J. K., and Di Stasi, L. C. (2011): Intestinal Anti-Inflammatory Activity of Baccharis dracunculifolia in the Trinitrobenzenesulphonic Acid Model of Rat Colitis. Evidence-based complementary and alternative medicine : eCAM, 52: 43-49.

Claiborne, A. (1985): Catalase activity. In: Greenwald RA (edsHandbook of Methods for Oxygen Radical Research Boca Raton, FL: CRC pp 283–284.

Das, K.M., Eastwood, M.A., McManus, J.P.A. and Sircus,W. (1973): Adverse reactions during salicylazosulfapyridine therapy and the relation with drug metabolism and acetylator phenotype. New England Journal of Medicine, 289: 491–495.

Dirlik, M., Karahan, A., Canbaz, H., Caglikulekci, M., Polat, A., Tamer, L., and Aydin, S. (2009): Effects of sulfasalazine on lipid peroxidation and histologic liver damage in a rat model of obstructive jaundice and obstructive jaundice with lipopolysaccharide-induced sepsis. Current therapeutic research, clinical and experimental, 70(4): 299–315.

Ekbom, A., Helmick, C., and Zack (1990): Ulcerative colitis and colorectal cancer: A population-based study. N Engl J Med, 323: 1228-1233.

Feranchak, A.P. and Sontag, M.K., et al. (1999): Prospective, long-term study of fat-soluble vitamin status in children with cystic fibrosis identified by newborn screen. J Pediatr., 135: 601-610.

Hoffmann, J. C., Pawlowski, N. N., Kühl, A. A., Höhne,W. and Zeitz, M. (2002): Animal models of inflammatory bowel disease: an overview. Pathobiology, 70: 121- 130.

Hossam, M.M., Ramadan, A., Hemeida, Ali, I.M. and Farid, M.A. (2009): Prophylactic role of Curcumin in dextran sulphate sodium(DSS) induced ulcerative colitis murine model. Food and Chem Toxicol, 47: 1311-1317.

Isozaki, Y., Yoshida, N., Kuroda, M., et al. (2006): Effect of a novel water-soluble vitamin E derivative as a cure for TNBS-induced colitis in rats. Int J Mol Med, 17: 497-502.

Hagar, H. H., El-Medany, A., El-Eter, E., and Arafa, M. (2007): Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats. European journal of pharmacology, 554(1): 69–77.

Hajj, H.I., Tohme, R., Barada, K., Mostafa, M.H., Freund, J.N., Jurjus, R.A., Karam, W. and Jurjus, A. (2008): Inflammatory bowel disease in rats: bacterial and chemical interaction. World J. Gastroenterol, 14: 4028-4039.

Jewell, D.P. and Patel, C. (1985): Immunology of inflammatory bowel disease. Scan. J. Gastroenterol., 114: 119-126.

Jurjus, A.R., Khoury, N.N. and Reimund, J.M. (2004): Animal models of inflammatory bowel disease. J. Pharmacol. Toxicol, Methods 50: 81-92.

Keshavarzian, A., Morgan, G., Sedghi, S., Gordon, J.H. and Doria, M. (1990): Role of reactive oxygen metabolites in experimental colitis. Gut, 31: 786-790.

Kitahora, T., Suzuki, K., Asakura, H., Yoshida, T., Suematsu, M.,Watanabe, M., Aiso, S. and Tsuchiya,M. (1998): Active oxygen species generated by monocytes and polymorphonuclear cells in patients in Crohn's disease. Dig. Dis. Sci. 33: 951–955.

Krawitz, J.E., Sharon, P. and Stenson, F. (1984): Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology, 87: 1344–1350.

Kruidenier, L. and Verspaget, H.W. (2002): Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease- radicals or ridiculous. Alimentary Pharmacology and Therapeutics, 16: 1997–2015.

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

Martın, M.C., Martinez, A., Mendoza, J.L., Taxonera, C., Dıaz-Rubio, M. and Fernandez-Arquero, M. (2007): Influence of the inducible nitric oxide synthase gene (NOS2A) on inflammatory bowel disease susceptibility. Immunogenetics, 59(11): 833–837.

Medhi, B., Prakash, A., Avti, P.K., Saikia, U.N., Pandhi, P. and Khanduja, K.L. (2008): Effect of Manuka honey and sulfasalazine in combination to promote antioxidant defense system in experimentally induced ulcerative colitis model in rats. Indian journal of experimental biology, 46(8): 583–590.

Michel, C., Bernard, C. and Lahaye, M. (1999): Algal oligosaccharides as functional foods: in vitro study of their cellular and fermentative effects; Les oligosides algaux comme aliments fonctionnels: etude in vitro de leurs effects cellularies et fermantaires. Sciences des aliments, 19: 311-332

Miles, A.M. and Grisham, M.B. (1994): Antioxidant properties of aminosalicylates. Methods in Enzymology, 234: 555–572.

Miranda, K., Espy, M.G. and Wink, D.A. (2001): A rapid and simple spectropho- tometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5: 62–71.

Mirbagheri, S.A., Nezami, B.G., Assa, S., et al. (2008): Rectal administration of d-alpha tocopherol for active ulcerative colitis: a preliminary report. World J Gastroenterology, 14: 5990-5995.

Morris, G.P., Beck, P.L., Herridge, M.S., et al. (1989): Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology, 96: 795-803.

Munoz, S.J., Heubi, J.E., et al. (1989): Vitamin E deficiency in primary biliary cirrhosis: gastrointestinal malabsorption, frequency and relationship to other lipid-soluble vitamins. Hepatology, 9: 525-531.

Mustafa, A., El-Medany, A., Hagar, H. H. and El-Medany, G. (2006): Ginkgo biloba attenuates mucosal damage in a rat model of ulcerative colitis. Pharmacological research : the official journal of the Italian Pharmacological Society, 53(4): 324–330.

Neurath, M. F., Fuss, I., Kelsall, B. L., Stüber, E. and Strober,W. (1995): Antibodies to interleukin 12 abrogate established experimental colitis in mice. J. Exp. Med, 182: 1281-1290.

Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y. and Nakaya, R. (1990): A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology, 98: 694-702.

Peppercorn, M.A. and Goldman, P. (1972): The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J Pharmacol Exp Ther, 181(3): 555–562.

Peppercorn, M.A. (1984): Sulfasalazine. Pharmacology, clinical use, toxicity, and related new" drug development. Ann. Intern. Med, 3: 377-386.

Plosker, G.L. and Croom, K.F. (2005): Sulfasalazine. A review of its use in the management of rheumatoid arthritis. Drugs, 65: 1825-1849

Podolsky, D.K. (1991): Inflammatory bowel disease. N. Engl. J. Med, 325: 1008–1016.

Rachmilewitz, D., Okon, E. and Karmeli, F. (1997): Sulphydryl blocker induced small intestinal inflammation in rats: a new model mimicking Crohn's disease. Gut, 41: 358- 365.

Satoh, H., Sato, F., Takami, K. and Szabo, S. (1997): New ulcerative colitis model induced by sulfhydryl blockers in rats and the effects of antiinflammatory drugs on the colitis. Jpn. J. Pharmacol. 73: 299-309.

Satok, H. and Szabo, S. (1990): New animal model of ulcerative colitis induced by sulfhudryl blockers in rats. Gastroenterology, 98: 202.

Schultz, J. and Kaminker, K. (1962): Myeloperoxidase of the leucocyte of normal human blood. I. Content and localization. Arch Biochem Biophys, 96: 465–467.

Sedlak, J. and Lindsay, R. H. (1968): Estimation of total, protein bond and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem, 25: 192-205.

Son, M., Ko, J.I., Kim, W.B., Kang, H.K. and Kim, B.K. (1998): Taurine can ameliorate inflammatory bowel disease in rats. Adv. Exp. Med. Biol., 442: 291–298.

Strober, W., Ludviksson, B.R. and Fuss, I.J. (1998): The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn's disease. Ann. Intern. Med, 128: 848–856.

Tahan, G., Aytac, E., Aytekin, H., Gunduz, F., Dogusoy, G., Aydin, S. and Uzun, H. (2011): Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid – induced ulcerative colitis in rats. Canadian Medical Association, 54(5): 333–338.

Tappel, A. and Zalkin, H. (1960): Inhibition of lipid peroxidation in microsomes by vitamin E. Nature, 185: 35.

Tolstanova, G., Deng, X., French, S. W., Lungo,W., Paunovic, B., Khomenko, T., Ahluwalia, A., Kaplan, T., Dacosta-Iyer, M., Tarnawski, A. (2012): Early endothelial damage and increased colonic vascular permeability in the development of experimental ulcerative colitis in rats and mice. Lab. Invest, 92: 9-21.

Uchiyama, Mitsuru, and Midori, M. (1978): Determination of Malonaldehyde Precursor in Tissues by Thiobarbituric Acid Test. Analytical Biochemistry 86: 271–278.

Viscido, A., Aratari, A., Maccioni, F., Signore, A. and Caprilli, R. (2005): Inflam- matory bowel diseases: clinical update of practical guidelines. Nucl Med Commun, 26: 649-655.

Wadie, W., Abdel-Aziz, H., Zaki, H. F., Kelber, O., Weiser, D., and Khayyal, M. T. (2012): STW 5 is effective in dextran sulfate sodium-induced colitis in rats. International journal of colorectal disease, 27(11), 1445–1453.

Witaicenis, A., Ana, C.L., Clélia, A.H., Sérgio, L.F., Natividad, G., Pilar, U., Julio, G. and Luiz, C. D. (2012): Suppression of TNBS-induced colitis in rats by 4-methylesculetin, a natural coumarin: comparison with prednisolone and sulphasalazine. Chemico-biological interactions, 195(1): 76–85.

Yamada, T. and Grisham, M.B. (1991): Role of neutrophil-derived oxidants in the pathogenesis of intestinal inflammation. Klinische Wochenschrift, 69: 988–994.

Zhou, Y.H., and Yu, J.P. (2007): Effects of Ginkgo biloba extract on the antioxidation in rats with TNBS-induced colitis. Shijie Hua- ren Xiaohua Zazhi, 15: 1701–1705.