J Gastroenterol 2005; 40:361–365
DOI 10.1007/s00535-005-1551-x

Journal of Gastroenterology

Lyprinol (stabilised lipid extract of New Zealand green-lipped mussel): a potential preventative treatment modality for inflammatory bowel disease

Danik Tenikoff[1,3], Karen J. Murphy[3,5], Maria Le[5], Peter R. Howe[3,5], and Gordon S. Howarth[1,2,3,4,5,6]

[1] Child Health Research Institute, Women's and Children's Hospital, North Adelaide, South Australia, Australia
[2] Gastroenterology Department, Women's and Children's Hospital, 72 King William Road, North Adelaide, SA 5006, Australia
[3] Discipline of Physiology, The University of Adelaide, Adelaide, South Australia, Australia
[4] Discipline of Paediatrics, The University of Adelaide, South Australia, Australia
[5] School of Pharmaceutical, Molecular and Biomedical Sciences, The University of South Australia, Adelaide, South Australia, Australia
[6] School of Biological, Sciences, Flinders University Bedford Park, Adelaide, South Australia, Australia

Background. Lyprinol (Pharmalink International), the stabilised lipid extract of the New Zealand green-lipped mussel, is currently used to relieve symptoms of arthritis. We investigated the effect of pretreatment with Lyprinol (LYP) on experimentally induced inflammatory bowel disease (IBD) in mice. ***Methods.*** Male C57BL/6 mice (aged 6 weeks) were gavaged daily for 13 days with (150 μl) olive oil (OO; $n = 7$), fish oil (FO; $n = 8$), or LYP ($n = 8$). Mice consumed 2% dextran sulfate sodium (DSS) for 6 days, starting on day 7. Body weight and disease activity index (DAI) scores were recorded daily. Colonic damage was determined by histopathology. Colonic inflammation was quantified by myeloperoxidase (MPO) activity. ***Results.*** LYP treatment significantly ($P < 0.05$) reduced body weight loss, DAI scores, crypt area losses, and cecum and colon weights, compared with FO treatment. MPO activity was not significantly affected by any treatment. ***Conclusions.*** These findings provide preliminary evidence that Lyprinol may be potentially useful in ameliorating symptoms of IBD. The benefit, however, is unlikely to be due to the omega-3 fatty acid content. Dose-response evaluation of Lyprinol in experimental IBD is warranted.

Key words: New Zealand green-lipped mussel, ulcerative colitis, fish oil, dextran sulfate sodium, mice

Introduction

Inflammatory bowel disease (IBD) is defined as a chronic, relapsing inflammation of unknown origin, with incidence rates of 4 to 10/100000 persons per year and prevalence rates between 40 and 100/100000 persons.[1–3] IBD comprises two conditions, known as Crohn's disease and ulcerative colitis. There is no known cure for IBD and the current treatments available fail to induce or maintain long-term remission in most patients.[1,2]

The persistence of the disease is strongly associated with relapsing inflammation of the intestine. Inflammatory mediators that prolong this chronic inflammation include the arachidonic acid metabolites leukotriene-B_4 (LTB_4), prostaglandin-E_2 (PGE_2), and thromboxane-B_2 (TXB_2), and the pro-inflammatory cytokines interleukin-1, -2, -6 (IL-1, IL-2, IL-6), tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-γ).[3] Treatment modalities, using corticosteroids and drugs such as sulfasalizine and 5-aminosalicylic acid, reduce intestinal inflammation and enhance colonic healing and repair of damaged tissue, in turn, reducing associated pain and swelling.[3] However, side-effects are commonly associated with the high doses of drugs required for therapy. Thus, there is a clear need for improved therapeutic approaches to improve the quality of life for patients.[1] Alternative treatments such as dietary omega-3 polyunsaturated fatty acids (n-3 PUFA), in the form of marine oils, have been demonstrated to reduce the symptoms of inflammation in a range of inflammatory diseases, including rheumatoid arthritis, psoriasis, and colitis. This effect was attributed to the n-3 PUFAs: eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3).[4]

One marine oil of particular interest is Lyprinol (Pharmalink International, Brisbane, Qld, Australia), the stabilized lipid extract from the New Zealand green-lipped mussel (NZGLM), *Perna canaliculus*, as it is thought to contain anti-inflammatory properties.[4–8] The oil of the NZGLM contains a complex mixture of triglycerides, sterol esters, sterols, polar lipids, and free fatty acids,[4,9,10] and has been shown to reduce the pro-inflammatory LTB_4 in human monocytes.[5] It is currently postulated that Lyprinol elicits an anti-inflammatory

Received: August 9, 2004 / Accepted: January 14, 2005
Reprint requests to: G.S. Howarth

effect, via EPA inhibition of both 5-lipoxygenase and cyclooxygenase arachidonate oxygenation pathways.[6] Furthermore, a recent human study has shown NZGLM lipids reduce levels of TXB_2, PGE_2, and IL-1β, with similar potency to low-dose n-3 PUFA supplementation.[4] Such findings support the potential for Lyprinol to reduce inflammation in IBD.

The objective of this study was to investigate the potential anti-inflammatory effects of Lyprinol (<1 mg n-3 PUFA/day) in a murine model of experimental ulcerative colitis. Using the dextran sulfate sodium (DSS)-induced model of colitis in male C57BL/6 mice, we assessed the effects of Lyprinol on colitic disease severity, compared to mice treated with fish oil (55 mg n-3 PUFA/day). We hypothesised that prophylactic administration of Lyprinol in this disease setting would reduce inflammation and, hence, the severity of disease.

Materials and methods

Animals and experimental design

The study followed the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the Animal Ethics Committee of Adelaide University and the Animal Care and Ethics Committee of the Women's and Children's Hospital, Adelaide.

Male C57BL/6 mice, aged 6 weeks (n = 23), were assigned to three treatment groups and administered 150 µl of either Lyprinol (LYP), fish oil (FO), or extra virgin olive oil (OO), via daily oral gavage, for 13 days. Lyprinol capsules (Pharmalink International) contained 50 mg NZGLM lipids, 100 mg natural monounsaturated olive oil, and 0.255 mg vitamin E (d-α-tocopherol). The daily dose of Lyprinol was a combination of 15 µl of the Lyprinol capsule diluted with 135 µl extra-virgin olive oil (Vetta; Meadow Lea Foods, Sydney, NSW, Australia). Thus each 150-µl gavage contained 5 mg NZGLM lipids, of which less than 1 mg comprised n-3 PUFA. This dose (5 mg/mouse) was selected on the basis of a previous study where Lyprinol was administered to adjuvant-induced polyarthritic rats at a dose of 20 mg/kg.[6] At this dose, Lyprinol was shown to be more anti-inflammatory than fish oil (90 mg/rat). Fish oil was prepared by combining oil from EPAX 5500 and EPAX 3000 capsules (EPAX 3000 + EPAX 5500 capsules; Pronova, Oslo, Norway). They were combined to comprise 210 mg EPA and 150 mg DHA per ml, equivalent to 55 mg EPA/DHA per 150 µl gavage. Following 7 days' prophylactic oil administration, DSS (ICN Biomedicals, Columbus, OH, USA) was dissolved in deionized water to a concentration of 2% (w/v) for oral consumption by mice for the remaining 6 days. This concentration of DSS was chosen based on previous work in our laboratory, using C57BL/6 mice which indicated that a moderate degree of colitis was manifest.[11] All mice were killed on day 13. The severity of colitis was assessed daily, using a disease activity index (DAI), based on a scoring system[12] which scored body weight loss, stool consistency, rectal bleeding, and overall condition, increasing in severity on a scale of 0–3 for each parameter. Overall condition was determined by two factors: (1) Mobility/agility: healthy mice are considered quite active, whereas mice affected by DSS characteristically become very weak and feeble, sitting hunched with very little movement. (2) Fur: healthy mice have well-groomed fur that lies flat to the body, whereas mice consuming DSS become scruffy. Body weight and water consumption were also recorded daily. Each mouse underwent the ^{13}C-octanoic acid breath-test on day 0 and day 13, following an overnight fast, for assessment of gastric half-emptying times ($t_{1/2}$) of a standard nutrient liquid, as a marker for potential upper gastrointestinal perturbation induced by the treatments.[13]

Collection of gut tissues

On day 13, mice were killed by CO_2 asphyxiation followed by cervical dislocation. Visceral organs were removed surgically as described previously.[14] Segments of proximal and distal colon (2 cm) were rapidly placed in 10% formalin fixative for histological analysis. The remaining ~1.5-cm mid-colon was frozen immediately in liquid nitrogen for future myeloperoxidase (MPO) determination. Finally, the other visceral organs, including the spleen, liver, thymus, lungs, heart, and kidneys, were weighed.

Tissue specimens for histological analysis were prepared by hematoxylin-and-eosin staining of 4-µm paraffin wax-embedded tissue sections and examined with a light microscope (Olympus BH-2, Tokyo, Japan). A semiquantitative histological assessment of intestinal damage was utilized to obtain an overall score of colitis severity.[15] Quantitative histological analysis was conducted on images acquired with a SONY digital camera (Tokyo, Japan) and measurements were performed using Image Pro Plus V.4.5.1.2.7 software (Media Cybernetics, MD, USA). Crypt area index was a ratio of the crypt area (µm^2) to total mucosal area (µm^2). We measured the area occupied by the crypts (= crypt area) and divided this by the total mucosal area. This ratio was then expressed as a percentage of the total mucosal area.

Biochemical myeloperoxidase (MPO) activity

MPO activity was used as a measure of neutrophil infiltration, and, hence, a determinant of inflammatory ac-

tivity. Samples of mid-colon (~5 cm) were homogenized (Heidolph homogenizer and type 10 G probe, Cinnaminson, NJ, USA) in potassium phosphate buffer containing 0.5% hexadecyltrimethyl-ammonium bromide (pH 6.0; 50 mg tissue per ml). After freeze-thawing the homogenate once, the tissue MPO activity was determined by previously described techniques.[16,17] Absorbance of each sample was determined at 450 nm by a microplate reader (Dynatech MR7000; Dynatech, Denkendorf, Germany). A unit of MPO activity was defined as that converting 1 µl of hydrogen peroxide to water in 1 min at 22°C.

Statistical analysis

The statistical tests were performed using Graph Pad InStat software (San Diego, CA, USA). We performed a one-way analysis of variance (ANOVA), which compared each group with the other, for statistical significance. If significance was seen ($P < 0.05$), post-hoc tests were performed using the Tukey-Kramer Post-test (for data where means ± SEM were measured). Where data was not normally distributed, non-parametric ANOVA was used (for data represented as median scores). If significance was seen ($P < 0.05$), post-hoc analyses were performed using the Kruskal-Wallis Post-test.

Results

Following administration of all oils, body weights increased rapidly from day 0 to day 1 due to recovery from a fasting period of approximately 20 h, prior to breath testing (Fig. 1). On day 1, LYP mice had a significantly higher body weight than FO-treated mice (Fig. 1; $P < 0.05$). Body weight gain reached a maximum on day 10 (17 ± 3%) for FO-treated mice, and day 11 (18 ± 2%) for OO-treated mice, and LYP-treated mice (22 ± 3%). Body weight began to decrease due to DSS consumption after maximum weight was reached. On day 12 of the trial, compared to the day-0 body weight, OO-treated mice had gained 15 ± 2% body weight, FO-treated mice had gained 6 ± 5%, while LYP-treated mice had gained 21 ± 3% (Fig. 1).

The DAI, indicated as [median (range)], in FO-treated mice (Fig. 2) was greater than that in LYP-treated mice on days 10, 11, 12, and 13 ($P < 0.05$). DAI in OO-treated mice was lower than that in FO-treated mice on day 10 only [OO, 0 (0–0); FO, 0.5 (0–2)]. There were no significant differences in DAI between LYP-treated mice or OO-treated mice on any day.

The crypt area index (CAI) of the distal colon was higher in LYP-treated mice (48.9 ± 9.4%), when compared to FO-treated mice (24.6 ± 9.0%; $P = 0.03$),

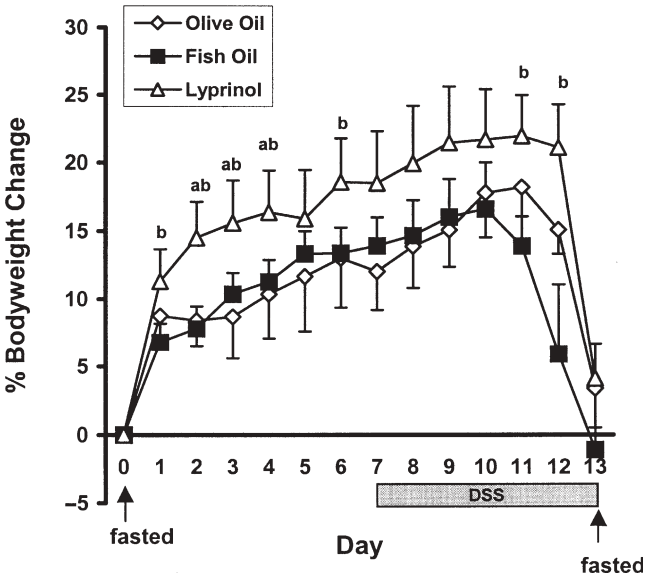

Fig. 1. Effect of daily oral administration of 150 µl of olive oil ($n = 7$), fish oil ($n = 8$), or Lyprinol (stabilised, lipid extract of New Zealand green-lipped mussel; Pharmalink International; $n = 8$) on the bodyweight change of C57BL/6 mice. Body weights were calculated as a percentage change from day 0. Mice ended a 15-h fasting period on days 0 and 13. Data values are expressed as means ± SEM. *a* Significantly different from olive oil ($P < 0.05$); *b* significantly different from fish oil ($P < 0.05$)

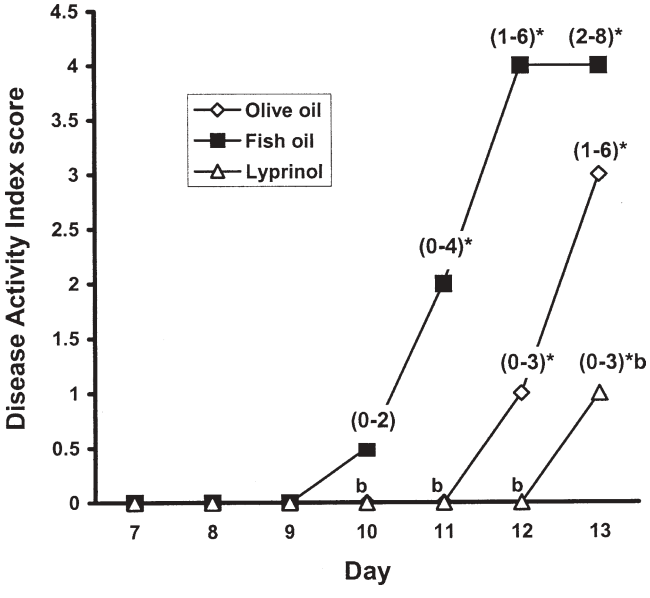

Fig. 2. Scoring of body weight loss, rectal bleeding, stool consistency, and overall condition was assessed by the disease activity index (DAI) and compared between mice treated with olive oil ($n = 7$), fish oil ($n = 8$), or Lyprinol ($n = 8$). Data are shown during the dextran sodium sulfate consumption period, which commenced on day 7. Data values are expressed as medians, with ranges indicated in parentheses. *b* significantly different from fish oil ($P < 0.05$); *, significantly different from day 0 ($P < 0.05$)

Table 1. Histological, physiological, and biochemical data from mice receiving 13 days' olive oil, fish oil, or Lyprinol (stabilised lipid extract of New Zealand green-lipped mussel; Pharmalink International) administration, at daily doses of 150 μl, commencing 7 days prior to oral consumption of 2% dextran sulfate sodium (DSS) for 6 days

Parameter (units)	Olive oil ($n = 7$)	Fish oil ($n = 8$)	Lyprinol ($n = 8$)	P value
Crypt area index (% total mucosal area)	39.8 ± 10.1	24.6 ± 9.0***	48.9 ± 9.4**	0.03
Colitis severity (median score)	5 (1–10)	9 (5–15)	4 (1–14)	0.07
Colon weight (mg)	154 ± 5	151 ± 7	133 ± 7*;***	0.05
Cecum weight (mg)	98 ± 7**	120 ± 12*;***	88 ± 7**	0.002

Data values are expressed as means ± SEM or medians (ranges). Significance was considered when $P < 0.05$, indicated by: *, significantly different from olive oil; **, significantly different from fish oil; ***, significantly different from Lyprinol

while OO-treated mice (39.8 ± 10.1%) were not significantly different from either group (Table 1). The colitis severity scores of the distal colon (Table 1) indicated a trend that supported the differences seen in CAI, in that FO-treated mice had the highest scores for colitis severity [OO, 5 (1–10); FO, 9 (5–15); LYP, 4 (1–14); $P = 0.07$]. The differences described in the distal colon did not extend to the proximal colon, where both crypt areas and colitis severity scores did not differ significantly between the three treatment groups (data not shown). Similarly, MPO activity, water consumption, and gastric emptying were not significantly affected by any treatment (data not shown).

On day 13, colon weight was decreased in LYP-treated mice (133 ± 7 mg) compared to both the OO (154 ± 5 mg) and FO (151 ± 7 mg) treatment groups (Table 1; $P = 0.05$). Cecum weights in LYP-treated (88 ± 7 mg) and OO-treated mice (98 ± 7 mg) were decreased in comparison to mice receiving FO (120 ± 12 mg; Table 1; $P < 0.05$).

Discussion

To date, this is the first study to investigate the potential anti-inflammatory effects of Lyprinol in experimentally induced IBD. Using the DSS-induced model of ulcerative colitis, we compared Lyprinol to fish oil, both with low doses of n-3 PUFA, while using extra-virgin olive oil as a control. In terms of body weight loss, DAI, and histopathologic damage, Lyprinol treatment was more effective than fish oil at reducing symptoms of DSS-induced colitis. However, since a sizeable component of Lyprinol comprised olive oil (90%), it was perhaps not surprising that the effects of Lyprinol were not marked at this dose.

In the current study, body weight loss was accompanied by diarrhea and blood loss as a result of damage to the colon and rectum, consistent with other studies of DSS-induced colitis.[14,18] Despite the significantly higher body weights on day 1 in Lyprinol-treated mice compared to fish oil-treated mice, it appeared that the onset of body weight loss, during the DSS consumption period, was delayed in Lyprinol-treated mice compared to fish oil-treated mice. Colitic disease activity was reduced in Lyprinol-treated mice, compared to fish oil treatment, presumably as a result of reduced tissue damage. The colon and cecum weights following Lyprinol treatment were lower than those after treatment with fish oil, which may have been an indication of reduced edema and inflammation, as both edema and the infiltration of inflammatory mediators could have contributed to increasing the weight of gastrointestinal organs. Crypt loss in the distal colon was reduced following Lyprinol treatment, as evidenced by greater crypt areas, compared to treatment with fish oil. Furthermore, the colitis severity scores of the distal colon further indicated that Lyprinol-treated mice suffered less tissue damage.

Previous reports regarding the anti-inflammatory mechanism of Lyprinol as being primarily n-3 PUFA-driven are not supported by the findings of this study, as fish oil contained higher levels of n-3 PUFA than Lyprinol, for a lesser effect. This minimal effect of fish oil may have been due to a lack of efficacy at such a low dose of n-3 PUFA, as it has been shown that fish oils with high doses of EPA and DHA exert anti-inflammatory effects.[5] Although the mechanism by which Lyprinol reduced DSS-induced colitis in the present study is unclear, it could be due to direct effects on T-lymphocyte function and/or cytokine production. Lyprinol contains a complex mixture of triglycerides, sterols, sterol esters, fatty acids, and polar lipids, as well as polyphenols, hydroxy fatty acids, and dl-α-tocopherol, as well as other unidentified compounds. Hence, it may be possible that these additional constituents were responsible for some of the effects seen. Similarly, a third of Lyprinol is olive oil, which is rich in polyphenols (oleuropein and hydroxytyrosol) and oleic acid (18:1n-9), and is thought to reduce risk factors for heart disease, lower cancer mortality, and reduce inflammation.[19–23] In animal studies, a diet feed contain-

ing 15% olive oil reduced carageenan-induced edema in rats.[24] In the present study, the marine oil formulations containing olive oil were unlikely to have had a major influence on the reduction of DSS-induced colitis; however, there may have been a synergistic effect with the olive oil components in addition to the Lyprinol components which could explain the lack of effect seen with fish oil (no olive oil). Further studies are warranted to investigate the mechanisms by which these compounds reduced the symptoms of DSS-induced colitis, including inflammatory marker (cytokine/eicosanoid) production.

In summary, we have shown that Lyprinol has the potential to reduce the severity and onset of IBD. The improvements seen in the present study in some indices of colitis compared with fish oil indicate that Lyprinol may have provided the potential to be more beneficial for treatment of colonic inflammation than fish oil. However, the optimal dose for Lyprinol administration may not have been achieved. Therefore, further studies are warranted to address the dose dependency of Lyprinol for maximum benefit against colitic disease, as well as the timing of administration, in order to determine its potential to form an adjunctive component to conventional IBD treatment.

References

1. Boismenu R, Chen Y. Insights from mouse models of colitis. J Leukoc Biol 2000;67:267–78.
2. Blumberg RS, Saubermann LJ, Strober W. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. Curr Opin Immunol 1999;11:648–56.
3. Hendrickson BA, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. Clin Microbiol Rev 2002;15:79–94.
4. Sinclair AJ, Murphy KJ, Li D. Marine lipids: overview "News insights and lipid compositions of Lyprinol™". Allerg Immunol (Paris) 2000;22:261–71.
5. Dugas B. Lyprinol® inhibits LTB4 production by human monocytes. Allerg Immunol (Paris) 2000;22:284–9.
6. Whitehouse MW, Macrides TA, Kalafatis N, Betts WH, Haynes DR, Broadbent J. Anti-inflammatory activity of a lipid fraction (Lyprinol) from The NZ Green-Lipped Mussel. Inflammopharmacology 1997;5:237–46.
7. Caughey DE, Grigor RR, Caughey EB, Young P. *Perna canaliculus* in the treatment of rheumatoid arthritis. Eur J Rheumatol Inflamm 1988;6:197–200.
8. Shiels IA, Whitehouse MW. Lyprinol: anti-inflammatory and uterine-relaxant activities in rats, with special reference to a model for dysmenorrhoea. Allerg Immunol (Paris) 2000;32:279–83.
9. Murphy KJ, Mooney BD, Mann NJ, Nichols PD, Sinclair AJ. Lipid, FA, and sterol composition of New Zealand Green Lipped Mussel (*Perna canaliculus*) and Tasmanian Blue Mussel (*Mytilus edulis*). Lipids 2002;37:587–95.
10. Murphy KJ, Mann NJ, Sinclair AJ. Fatty acid and sterol composition of frozen and freeze-dried New Zealand Green Lipped Mussel (*Perna canaliculus*) from three sites in New Zealand. Asia Pac J Clin Nutr 2003;12:50–60.
11 Geier MS, Abbott CA, Tenikoff D, McCaughan GW, Howarth GS. Development and resolution of experimental colitis in dipeptidyl peptidase IV knockout mice. J Cell Physiol 2004.DOI 10.1002/jcp.20333.
12. Murthy SN, Cooper HS, Shim H, Shah RS, Ibrahim SA, Sedergran DJ. Treatment of dextran sulphate sodium-induced murine colitis by intracolonic cyclosporin. Dig Dis Sci 1993;38: 1722–34.
13. Symonds EL, Butler RN, Omari TI. Assessment of gastric emptying in the mouse using the [^{13}C]-octanoic acid breath test. Clin Exp Pharmacol Physiol 2000;27:671–5.
14. Howarth GS, Xian CJ, Read LC. Insulin-like growth factor-I partially attenuates colonic damage in rats with experimental colitis induced by oral dextran sulphate sodium. Scand J Gastroenterol 1998;33:180–90.
15. Howarth GS, Francis GL, Cool JC, Xu X, Byard RW, Read LC. Milk growth factors enriched from cheese whey ameliorate intestinal damage by methotrexate when administered orally to rats. J Nutr 1996;126:2519–30.
16. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982;78:206-9.
17. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 1989;96:795–803.
18. Kitajima S, Takuma S, Morimoto M. Tissue distribution of dextran sulfate sodium (DSS) in the acute phase of murine DSS-induced colitis. J Vet Med Sci 1999;61:67–70.
19. Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, et al. Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncol 2000;1:107-12.
20. Patrick L, Uzick M. Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. Altern Med Rev 2001;6:248–71.
21. Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A prospective study of tomato products, lycopene, and prostate cancer risk. J Natl Cancer Inst 2002;94:391–8.
22. Nieto N, Torres MI, Rios A, Gil A. Dietary polyunsaturated fatty acids improve histological and biochemical alterations in rats with experimental ulcerative colitis. J Nutr 2002;132:11–9.
23. Visioli F, Galli C. Biological properties of olive oil phytochemicals. Crit Rev Food Sci Nutr 2002;42:209–21.
24. Martinez-Dominguez E, de la Puerta R, Ruiz-Gutierrez V. Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. Inflamm Res 2001;50:102-6.