

# ANNALS *of the* New York Academy of Sciences

## **Fermented Wheat germ Extract (Avemar) Inhibits Adjuvant Arthritis**

ANDRAS TELEKES, AKOS RESETAR, GEZA BALINT,  
GABOR BLAZSO, GYORGY FALKAY, KAROLY LAPIS,  
ERZSEBET RASO, BELA SZENDE, MICHAEL EHRENFELD,  
YEHUDA SHOENFELD, AND MATE HIDVEGI

**ABSTRACT:** Anti-inflammatory efficacy of the fermented wheat germ extract (FWGE, Avemar) in the rat adjuvant arthritis (AA) model was examined. To Wistar rats with AA, different doses of FWGE and antiinflammatory drugs (indomethacin, dexamethasone) as monotherapies were administered and FWGE and either diclofenac or dexamethasone were also given in combination. Besides plethysmographies of the paws, histological investigations of synovial tissues were also performed along with detection of CD4+ and CD8+ T lymphocytes. Gene expressions of COX-1 and 2 were determined by real-time polymerase chain reaction (PCR). FWGE monotherapy significantly inhibited the development of the secondary (immune-mediated) response in AA, and dexamethasone and indomethacin exerted inhibitory effects in a degree comparable to that of FWGE. Histological analysis of the affected joints confirmed the results. FWGE inhibited COX-1 and -2, while indomethacin enhanced COX-2 gene expressions. FWGE had an additive interaction with diclofenac. It is concluded that FWGE has significant anti-inflammatory efficacy confirmed by plethysmography, histology, and real-time PCR.

**KEYWORDS:** FWGE; Avemar; fermented wheat germ extract; adjuvant arthritis; anti-inflammatory effect; COX-1 inhibition; COX-2 inhibition

# Fermented Wheat Germ Extract (Avemar) Inhibits Adjuvant Arthritis

ANDRAS TELEKES,<sup>a,j</sup> AKOS RESETAR,<sup>b,j</sup> GEZA BALINT,<sup>c</sup>  
GABOR BLAZSO,<sup>d</sup> GYORGY FALKAY,<sup>d</sup> KAROLY LAPIS,<sup>e</sup>  
ERZSEBET RASO,<sup>f</sup> BELA SZENDE,<sup>e</sup> MICHAEL EHRENFELD,<sup>g</sup>  
YEHUDA SHOENFELD,<sup>h</sup> AND MATE HIDVEGI<sup>i</sup>

<sup>a</sup>*Chemotherapy Day-Ward, National Institute of Oncology, Budapest, Hungary*

<sup>b</sup>*Biomedicina First Hungarian Corporation for Cancer Research and Oncology, Budapest, Hungary*

<sup>c</sup>*National Institute of Rheumatology and Physiotherapy, Budapest, Hungary*

<sup>d</sup>*Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary*

<sup>e</sup>*First Institute of Pathology and Experimental Cancer Research, Faculty of Medicine, Semmelweis University, Budapest, Hungary*

<sup>f</sup>*Department of Tumor Progression, National Institute of Oncology, Budapest, Hungary*

<sup>g</sup>*Department of Medicine C, Sheba Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Hashomer, Israel*

<sup>h</sup>*Department of Medicine B, and Center for Autoimmune Diseases, Sheba Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Hashomer, Israel*

<sup>i</sup>*Jewish University, Budapest, Hungary*

**ABSTRACT:** Anti-inflammatory efficacy of the fermented wheat germ extract (FWGE, Avemar) in the rat adjuvant arthritis (AA) model was examined. To Wistar rats with AA, different doses of FWGE and anti-inflammatory drugs (indomethacin, dexamethasone) as monotherapies were administered and FWGE and either diclofenac or dexamethasone were also given in combination. Besides plethysmographies of the paws, histological investigations of synovial tissues were also performed along with detection of CD4+ and CD8+ T lymphocytes. Gene expressions of COX-1 and 2 were determined by real-time polymerase chain reaction (PCR). FWGE monotherapy significantly inhibited the development of the secondary (immune-mediated) response in AA, and dexamethasone and indomethacin exerted inhibitory effects in a degree comparable to

Address for correspondence: Prof. Yehuda Shoenfeld, M.D., F.R.C.P. (Hon.), Head, Department of Medicine B and Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer 52621, Israel. Voice: +972-3-530-2652; fax: +972-3-535-2855.

Yehuda.Shoenfeld@sheba.health.gov.il

<sup>j</sup>Drs. Telekes and Resetar contributed equally to this work.

Ann. N.Y. Acad. Sci. 1110: 348–361 (2007). © 2007 New York Academy of Sciences.  
doi: 10.1196/annals.1423.037

that of FWGE. Histological analysis of the affected joints confirmed the results. FWGE inhibited COX-1 and -2, while indomethacin enhanced COX-2 gene expressions. FWGE had an additive interaction with diclofenac. It is concluded that FWGE has significant anti-inflammatory efficacy confirmed by plethysmography, histology, and real-time PCR.

**KEYWORDS:** FWGE; Avemar; fermented wheat germ extract; adjuvant arthritis; anti-inflammatory effect; COX-1 inhibition; COX-2 inhibition

## INTRODUCTION

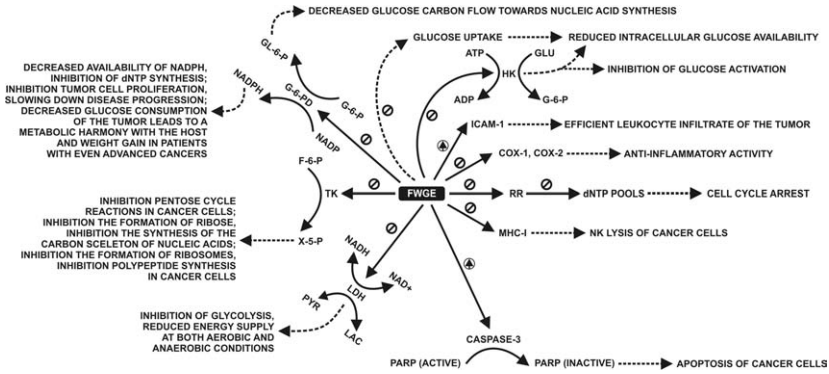
A fermented wheat germ extract (FWGE, code name: MSC, trade name: Avemar), standardized to and characterized by its methoxy-substituted benzoquinone contents and by its qualitative high-performance liquid chromatographic spectra, was developed in Hungary in the early 1990s. In some European countries, this orally applicable preparation has been approved as an over-the-counter medical nutriment (dietary food for special medical purpose) for cancer patients, because it has been demonstrated to have supportive therapeutic effects in the treatment of different human neoplastic diseases.<sup>1-5</sup> Although the specific molecule or molecules of the extract responsible for its beneficial health effects has not been identified yet, the molecular targets of this dietary food for special medical purposes are, at least partially, known (Fig. 1).<sup>6-11</sup> In a pilot clinical study it has been observed that FWGE, when administered together with anti-inflammatory drugs in patients with severe rheumatoid arthritis (RA), could significantly improve disease outcome.<sup>12</sup> It was therefore decided to find out more of the underlying mechanisms by which this extract can set forth these positive effects in RA.

Wistar rat adjuvant arthritis (AA) is still a relevant animal model of human RA<sup>13</sup> and also a widely used chronic model for studying the anti-inflammatory properties of drugs.<sup>14</sup> The onset of symptoms in the noninjected paw (e.g., “secondary” or “immune-mediated” response, which is considered to be the model of RA) occurs between the 8th–12th day and the disease approaches maximal intensity between days 16 and 21 after the injection of the adjuvant.<sup>15</sup> The aim of our study was to investigate the mechanism of action of the FWGE in the rat AA model.

## MATERIALS AND METHODS

### *Animals*

Female Wistar rats (weighing  $200 \pm 5$  g) were used. The animals were kept in plastic cages (max. 5 rats per cage) under controlled illumination (lights on from 07:00–19:00 h), fed with standard rodent food pellets (LATI, Gödöllő,



**FIGURE 1.** Molecular targets of the fermented wheat germ extract (FWGE). By the activation (⊕) of the caspase-3 downstream proteases, FWGE induces cleavage of poly(ADP-ribose) polymerase (PARP), which leads to apoptosis of cancer cells. FWGE downregulates the major histocompatibility complex class I (MHC-I) proteins on tumor cells' surface, thus making them targets of natural killer (NK) cells. The extract inhibits (⊖) the activity of ribonucleotide reductase (RR), the key enzyme of *de novo* DNA synthesis. FWGE also inhibits cyclooxygenase (COX)-1 and -2 and thus has anti-inflammatory activity. It has also been shown that FWGE upregulates the expression of intercellular adhesion molecule-1 (ICAM-1) on the endothelial cell. It is known that endothelial cells of the vasculature of human solid tumors have a decreased expression of ICAM-1 compared to normal endothelial cell tissue, and this phenomenon can be considered as a tumor-derived escape mechanism since the development of an efficient leukocyte infiltrate of the tumor is impaired. FWGE decreases glucose (GLU) uptake both directly and by inhibiting glucose activation via the inhibition of hexokinase (HK), the catalyst of activation by phosphorylation. FWGE also inhibits pentose cycle enzymes involved in direct glucose oxidation (glucose-6-phosphate dehydrogenase, G-6-PD) and nonoxidative glucose utilization (transketolase, TK) toward nucleic acid synthesis. These inhibitions result in decreased glucose consumption of cancer cells and thus the progression of the neoplastic disease slows down. The extract further inhibits the enzyme lactate dehydrogenase (LDH), which results in decreased glycolytic flux and reduced energy supply of tumor growth at both aerobic and anaerobic conditions. In contrast, FWGE treatment is about 50× less effective in peripheral blood lymphocytes in inducing biological effects, which provides a comfortable therapeutic window for FWGE to apply in patients as a supplemental treatment modality with minimal or no toxic side effects. Further abbreviations: dNTP = deoxyribonucleoside triphosphate; ATP = adenosine 5'-triphosphate; ADP = adenosine 5'-diphosphate; G-6-P = glucose-6-phosphate; GL-6-P = glucono lactone-6-phosphate; F-6-P = fructose-6-phosphate; X-5-P = xylulose-5-phosphate; NAD<sup>+</sup>, NADH = nicotinamide adenine dinucleotide, oxidized and reduced forms, respectively; NADP, NADPH = nicotinamide adenine dinucleotide phosphate, oxidized and reduced forms, respectively; PYR = pyruvate; LAC = lactate.

Hungary) and given tap water *ad libitum*. The room temperature and the relative humidity were  $23 \pm 1^\circ\text{C}$  and  $50 \pm 5\%$ , respectively. The experimental protocols had been approved by an institutional expert board (Ethical Committee for Preventing Abuse and Torture of Animals).

### *Induction and Evaluation of the Degree of AA*

The rats were injected subcutaneously in the right hind footpad with 0.5 mg of heat-killed, desiccated, homogenized *Mycobacterium butyricum* (Difco, Detroit MI, USA) in 0.1 mL of liquid paraffin. Prior to injection of the adjuvant and then 1, 4, 7, 12, 14, 18, and 22 days later, the volumes of both (right = injected and left = noninjected) hind paws (the measure of the primary and secondary reaction, respectively) were determined by plethysmography with the animal under light ether anesthesia. The inhibition of inflammation was calculated by comparing the volumes of the legs to those of the control as 100%.

### *Chemicals*

FWGE (Biomedicina, Budapest, Hungary) in different doses, and positive controls (indomethacin [Chinoin-Sanofi, Budapest, Hungary], diclofenac-Na [Sigma-Aldrich, Budapest, Hungary], and dexamethasone phosphate [MSD, West Point WA, USA]) were administered orally (by gastric tube) twice a day (i.e., between 8:30 and 10:30 and between 16:30 and 18:30 h). FWGE and dexamethasone were suspended in distilled water, while indomethacin and diclofenac were suspended in 0.5% carboxymethylcellulose, and all treatments were administered at a dose of 1.0 mL/150 g body weight.

### *Experimental Design*

In the first set of experiments two arms (A and B) were created. In Arm A FWGE, the positive controls, and placebo were administered *per os* 24 h prior to the adjuvant subplantar injection (in rats weighing  $138 \pm 5$  g). In Arm B treatments were initiated 14 days prior to the adjuvant subplantar injection (in rats weighing  $118 \pm 5$  g). Both arms consisted of seven groups: control ( $2 \times 1$  mL/150 g distilled water); FWGE  $2 \times 2.5$ ;  $2 \times 1.0$ ;  $2 \times 0.25$  and  $2 \times 0.05$  g/kg/day; indomethacin  $2 \times 0.5$  mg/kg/day; dexamethasone  $2 \times 50.0$   $\mu$ g/kg/day. Each experimental group consisted of 10–16 rats. Histologic examination was performed on rats sacrificed on day 21.

In the second set of experiments (confirmatory study; rats weighing  $130 \pm 10$  g) the study, was done in two steps (dose-finding for equal anti-inflammatory effects and, interactional). *Dose-finding*: control ( $2 \times 1$  mL/150 g distilled water); FWGE  $2 \times 2.5$  and  $2 \times 1.0$  g/kg/day; diclofenac  $2 \times 0.3$ ;  $2 \times 1.0$ ;  $2 \times 3.0$ ; and  $2 \times 10.0$  mg/kg/day; dexamethasone  $2 \times 1.0$ ;  $2 \times 3.0$ ;  $2 \times 10.0$ ; and  $2 \times 30.0$   $\mu$ g/kg/day. Each group consisted of 10 rats. On day 15, two rats of each group were sacrificed for histologic study. *Interactional*: control ( $2 \times 1$  mL/150 g distilled water); FWGE  $2 \times 2.5$  g/kg/day; diclofenac  $2 \times 1.0$  mg/kg/day; dexamethasone  $2 \times 15.0$   $\mu$ g/kg/day; FWGE  $2 \times$

1.25 g/kg/day + diclofenac  $2 \times 0.5$  mg/kg/day; FWGE  $2 \times 1.25$  g/kg/day + dexamethasone  $2 \times 7.5$   $\mu$ g/kg/day; FWGE  $2 \times 1.0$  g/kg/day; diclofenac  $2 \times 0.4$  mg/kg/day; dexamethasone  $2 \times 6.0$   $\mu$ g/kg/day; FWGE  $2 \times 0.5$  g/kg/day + diclofenac  $2 \times 0.2$  mg/kg/day; FWGE  $2 \times 0.5$  g/kg/day + dexamethasone  $2 \times 3.0$   $\mu$ g/kg/day. The rats were sacrificed by exsanguination under Nembutal narcosis on day 24 after the adjuvant injection.

### *Histologic Studies*

The affected joints of the left hind footpad together with the epiphyses of the bones and the surrounding fibrous and muscular tissues were fixed in buffered neutral 4% formalin. Decalcification was performed using EDTA and the samples were embedded into paraffin. Eight  $\mu$ m thin longitudinal sections were cut and the sections were stained with hematoxylin and eosin (H+E). In selected positive control and treated cases immunoperoxidase reaction was performed to demonstrate CD4+ and CD8+ T lymphocytes. The antibodies (Santa Cruz Biochemicals, Santa Cruz CA, USA) were applied at 1:100 dilution.

### *COX-Genes Expression*

Real-time polymerase chain reaction (PCR) was carried out to determine gene expressions of COX-1 and COX-2. Whole blood was collected from the aorta into EDTA-containing tubes to prevent coagulation. The samples were lysed with red blood cell lysing buffer (Sigma-Aldrich) and total RNA was extracted from the remaining white blood cells using Tri-Reagent (Sigma-Aldrich) according to the manufacturer's protocol. Purity of the nucleic acid templates was elevated by extrapurification with DNA-free<sup>TM</sup> (Ambion, Austin TX, USA). One microgram of total RNA was reverse-transcribed from each sample using deoxy (d)-NTPs (0.5 mmol/L each) random primer (final concentration 3  $\mu$ M), RNasin<sup>TM</sup> ribonuclease inhibitor (Promega, Madison WI, USA) (0.4 U/ $\mu$ L), DTT (final concentration 10 mM), reverse transcriptase buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, and 15 mM MgCl<sub>2</sub>) and M-MLV reverse transcriptase (Sigma-Aldrich) (200 U/reaction). Samples were incubated for 50 min at 37°C and then at 85°C for 10 min. Primers for quantitative PCR were designed using the Array Designer (Premier Biosoft International, Palo Alto CA, USA). The sequences of COX-1 primers were: 5'-GTC TGA TGC TCT TCT CCA CGA-3' and 5'-ATG AAC GGA TGC CAG TGA TAG A-3'. The sequences of COX-2 primers were: 5'-GTG GTG AAT GTA TGA GCA TAG GAT-3' and 5'-GTG TAG TAG GAG AGG TTG GAG AA-3'. The real-time PCR analysis for mRNA expression of COX-1 and COX-2 was standardized by coamplifying these genes with the housekeeping gene  $\beta$ -actin (primers were: 5'-GTG GGG CGC CCC AGG CAC CCA-3' and

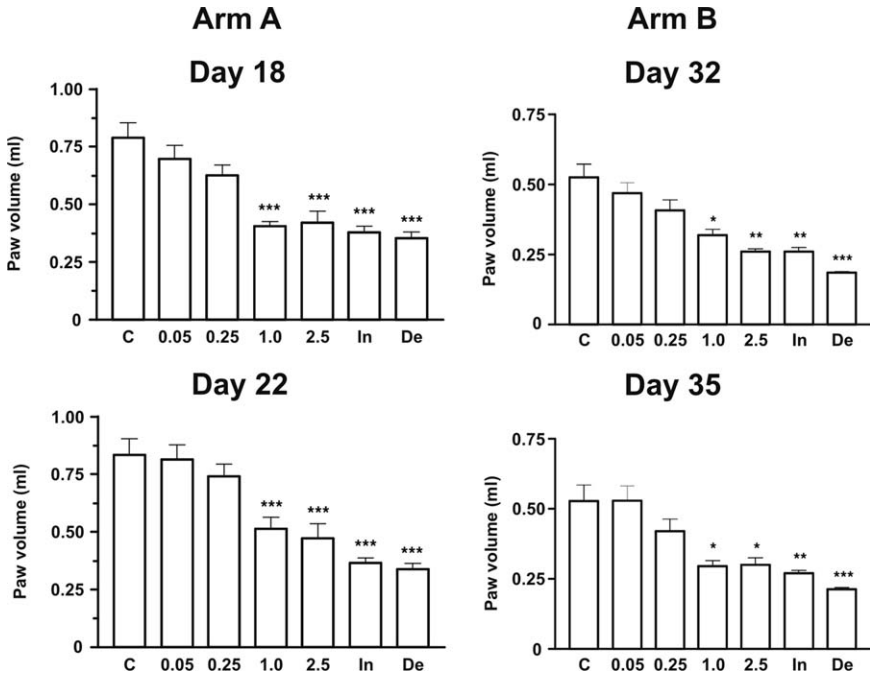
5'-GTC CTT AAT GTC ACG CAC GAT TTC-3'). The real-time PCR reaction was run on the iCycler iQ™ (Bio-Rad, Hercules CA, USA) using standard conditions: optimized concentration of primers (final concentration: 200 nM), IQ™SYBR™ Green Supermix (Bio-Rad) (100 mM KCl, 40 mM Tris-HCl, pH 8.4, 0.4 nM of each dNTP, 6 mM MgCl<sub>2</sub>, 50 U/mL iTaq DNA polymerase, SYBR Green I, 20 nM fluorescein) and 2 μL cDNA. The starting quantity of gene expression in the sample was determined by comparison with a standard curve generated from a dilution series of template DNA (rat Wilms' tumor, RWT) of known concentration (6.25e<sup>2</sup>, 1.25e<sup>2</sup>, 2.5e, 5.0, 1.0, where e is the base of the natural logarithm). A no-template control (containing water) was used as a negative control for every different primer pair. Relative gene expression was calculated by dividing the starting quantity of gene expression (normalized to the housekeeping gene) in treated (indomethacin, dexamethasone, and three different FWGE doses) rats by that in the untreated controls.

### *Statistical Analysis*

Statistical analyses were performed by Prism 2.01 software (GraphPad, San Diego CA, USA). Differences in paw volume or body weight were analyzed with one-way analysis of variance (ANOVA) followed by Neuman–Keuls *post hoc* test. Differences were considered significant at  $P < 0.05$ . Data are given as means  $\pm$  SE.

## RESULTS

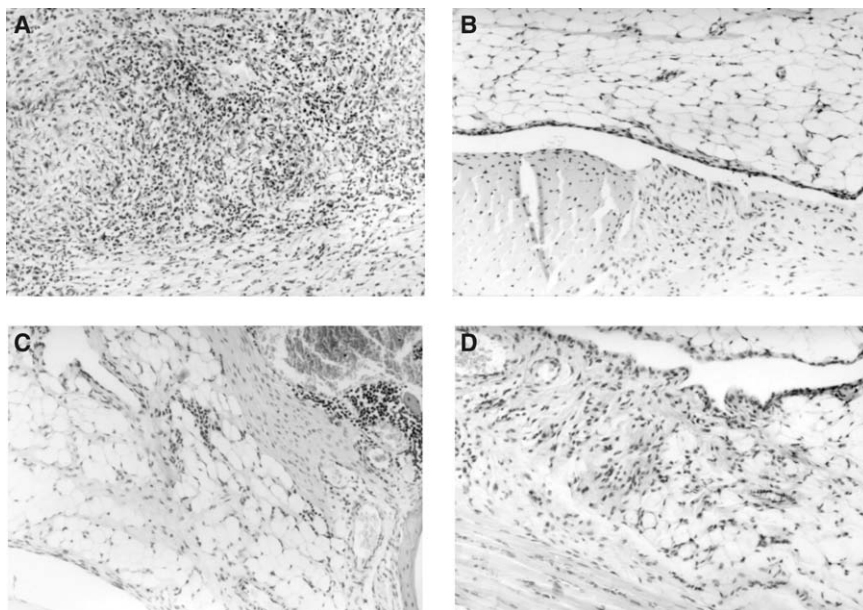
In the first set of experiments the effects of FWGE, indomethacin, and dexamethasone on the injected and noninjected paw volume, and histology of the joints were examined. Small oral doses of FWGE ( $2 \times 0.05$  and  $2 \times 0.25$  g/kg/day) did not influence the progression of AA. However, treatment with higher dosages ( $2 \times 1.0$  and  $2 \times 2.5$  g/kg/day) significantly suppressed the swelling and inhibited the development of the “secondary” (immune-mediated) response in the left paw. Treatments with positive control reference drugs like dexamethasone ( $2 \times 50.0$  μg/kg/day) or indomethacin ( $2 \times 0.5$  mg/kg/day) also effectively suppressed the swelling and the appearance of the immune-mediated response. Statistically significant anti-inflammatory activity of the FWGE was determined on days 14, 18, or 22 after the subplantar injection of *Mycobacterium butyricum* in the right hind paw. FIGURE 2 shows the effects of treatments on the noninjected paws (considered to be the model of immune-mediated arthritis) on days 18 and 22 in arm A of the study, and on days 32 and 35 in arm B. It is important to note that days 32 and 35 in arm B correspond to days 18 and 22 (following the initiation of AA) in arm A, respectively. There were no statistically significant differences between the AA-inhibitory



**FIGURE 2.** Anti-inflammatory efficacies of different doses of oral FWGE treatment in AA at days 18 and 22 (arm A) and 32 and 35 (arm B). Higher doses of FWGE ( $2 \times 1.0$ – $2 \times 2.5$  g/kg/day) significantly inhibited the immune-mediated inflammation in both arms. Note that the efficacy of FWGE is comparable to that of indomethacin (In) or dexamethasone (De). Day 32 and 35 in arm B (14 days, pretreatment) correspond to day 18 and 22 in arm A (24-h pretreatment), respectively. C = control. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

efficacies of the 24 h (arm A) or 14 day (arm B) pretreatments. The efficacies of treatments on the injected paws were also detected, but were not as obvious as on the noninjected paws (data are not shown).

Histological examinations of the joints of the left hind paws in untreated control rats suffering from AA revealed severe inflammatory changes of the synovium and surrounding tissues (FIG. 3A). The cellular infiltration consisted of lymphocytes, plasma cells, histiocytes, multinucleated giant cells, and fibroblasts. Microabscesses formed by neutrophil granulocytes were also seen occasionally. The majority of the lymphocytes proved to be CD4-positive. The joints of the FWGE (1.0 and 2.5 g/kg/day)-treated rats showed no or minimal inflammatory infiltration with almost complete disappearance of CD4-positive lymphocytes and minimal degree of fibrosis (FIG. 3B). The same applies for the indomethacin- (FIG. 3C) and the dexamethasone (FIG. 3D)-treated animals. The histology of synovial membrane of the rats treated with lower doses of FWGE did not differ significantly from the untreated controls. No significant

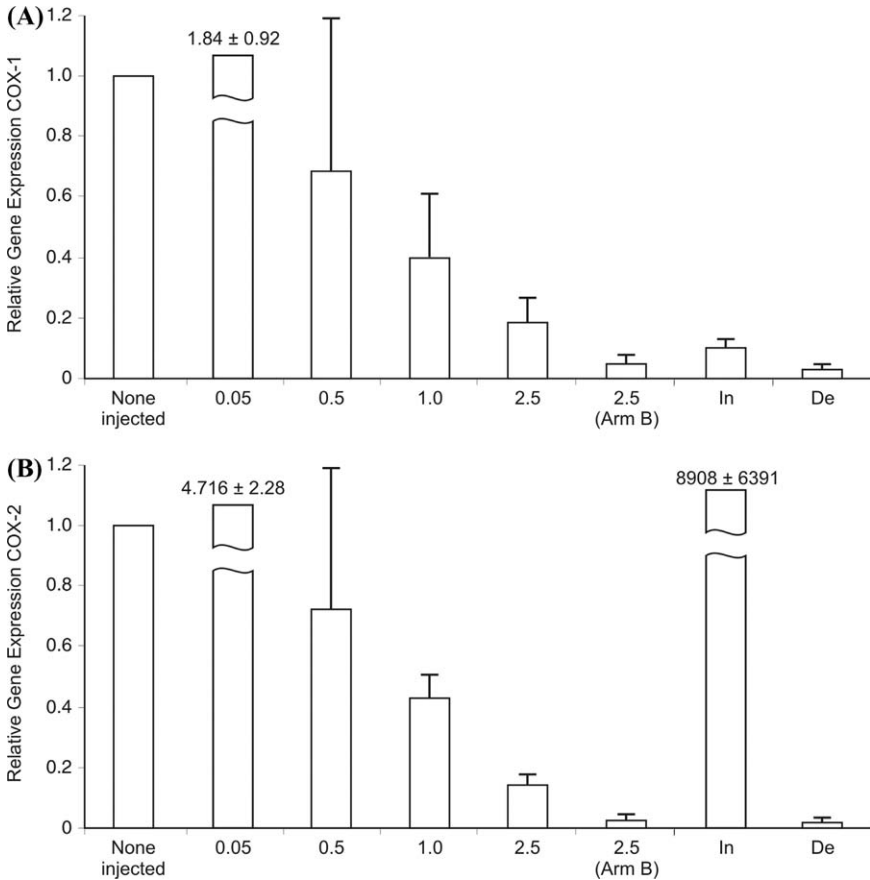


**FIGURE 3.** (A) Severe chronic inflammatory infiltration of the synovium and surrounding tissues of an untreated AA rat. Twenty-four hours pretreatment. (H and E,  $\times 300$ ). (B) Lack of inflammatory infiltrate in the synovial and perisynovial tissue of an AA rat treated by  $2 \times 2.5$  g/kg/day FWGE. Twenty-four hours pretreatment. (H and E,  $\times 300$ ). (C) Minimal fibrosis in the synovial and perisynovial tissue of an AA rat, treated by  $2 \times 0.5$  mg/kg/day indomethacin. Twenty-four hours pretreatment. (H and E,  $\times 300$ ). (D) Minimal cellular infiltration in the synovial and perisynovial tissue of an AA rat, treated by  $2 \times 50.0$   $\mu$ g/kg/day dexamethasone. Twenty-four hours pretreatment. (H and E,  $\times 300$ ).

difference was found between the groups receiving 24-h (arm A) or 14-day (arm B) pretreatments.

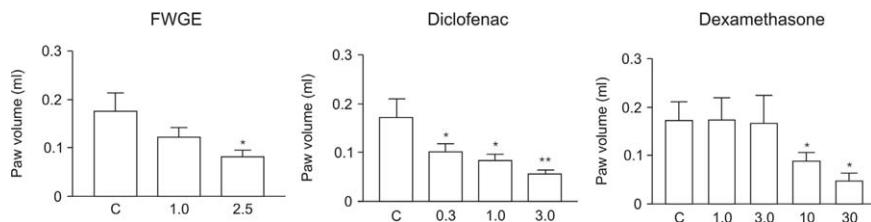
FWGE had a dose-dependent inhibitory activity on relative gene expression of both COX-1 and COX-2 (Fig. 4). The same doses of FWGE induced similar degree of inhibition both in COX-1 and COX-2. It is noteworthy that the lowest doses of FWGE had inverse activity: slightly stimulated COX-1 and COX-2 expressions. The 14-day pretreatments enhanced COX-1 and COX-2 inhibition: the pretreated groups showed further improvement. The relative gene expressions in arms A and B for COX-1/COX-2 expression were  $0.185 \pm 0.09/0.051 \pm 0.042$  and  $0.135 \pm 0.063/0.022 \pm 0.018$ , respectively.

In the dose-finding part of the second set of experiments the dose-effect relationships of the treatments were investigated. In the left hind paws, FWGE at the dose of  $2 \times 1.0$  g/kg/day and  $2 \times 2.5$  g/kg/day inhibited the inflammation by 30% and 54%, respectively. In the left hind paws, diclofenac at the dose of  $2 \times 0.3$ , 1.0, and 3.0 mg/kg/day inhibited the inflammation by 42%, 53%, and 68%,



**FIGURE 4.** Relative COX-1 and COX-2 gene expressions in AA rats treated by different dosages of FWGE and by indomethacin (In) and dexamethasone (De). Fourteen days' pretreatment (Arm B) significantly enhanced the inhibition ( $P < 0.001$ ). The relative gene expression of COX-2 shows similar pattern to that of COX-1 by FWGE treatment. Indomethacin enhances the relative gene expression of COX-2 by 9,000 times.

respectively. The dose of diclofenac of  $2 \times 10.0$  mg/kg/day killed all the rats before the end of the planned treatment period. Dexamethasone at the dose of  $2 \times 1.0$ , 3.0, 10, and 30  $\mu$ g/kg/day inhibited the inflammation by 0%, 4%, 48%, and 72% cent, respectively (FIG. 5). In the study of interactions of the drugs (FIG. 6), FWGE ( $2 \times 1.0$  g/kg/day) and diclofenac ( $2 \times 0.4$  mg/kg/day) inhibited the inflammation by 26% and 38%, respectively, while the combination of the half doses (FWGE  $2 \times 0.5$  g/kg/day + diclofenac  $2 \times 0.2$  mg/kg/day) resulted in 38% inhibition. The higher dose of FWGE ( $2 \times 2.5$  g/kg/day) and



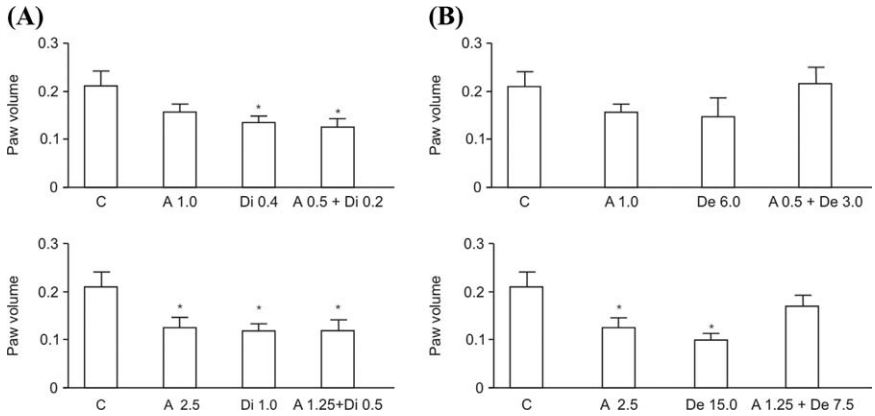
**FIGURE 5.** The dose-dependent anti-inflammatory efficacies of FWGE, diclofenac, and dexamethasone in AA. C: control. \* $P < 0.05$ ; \*\* $P < 0.01$ .

diclofenac ( $2 \times 1.0$  mg/kg/day) showed 40% and 43% inhibitory activities, respectively, and the combination of the half doses (FWGE  $2 \times 1.25$  g/kg/day + diclofenac  $2 \times 0.5$  mg/kg/day) resulted in 42% inhibition. Both FWGE ( $2 \times 1.0$  g/kg/day) and dexamethasone ( $2 \times 6$  µg/kg/day) resulted in a nonsignificant 28% inhibition and the combination of the half doses (FWGE  $2 \times 0.5$  g/kg/day + dexamethasone  $2 \times 3$  µg/kg/day) showed no inhibitory activities at all. The higher dose of FWGE ( $2 \times 2.5$  g/kg/day) and dexamethasone ( $2 \times 15$  µg/kg/day) resulted in significant inhibition of 40% and 50%, respectively, while the combination of the half doses (FWGE  $2 \times 1.25$  g/kg/day + dexamethasone  $2 \times 7.5$  µg/kg/day) showed 16% inhibition. In the injected paws, anti-inflammatory efficacies of all treatments were also detected, but these were less consistent than those in the noninjected paws (data are not shown).

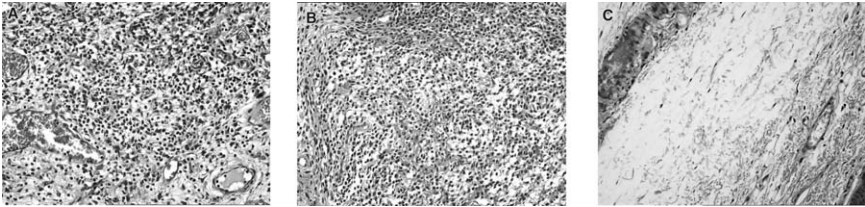
The solvent-treated controls showed severe inflammations in the left paws (FIG. 7A). FWGE treatment in a higher dose (2.5 g/kg/day) decreased the reaction to a minimal level and so did the 1.0 mg/kg/day diclofenac treatment. Combination of FWGE (1.25 g/kg/day) and diclofenac (0.5 mg/kg/day) resulted in a weak inflammatory reaction characterized only by scanty lymphocytic infiltration. However, 15 µg/kg dexamethasone as a single therapy could only moderately decrease the inflammation, and the combination of dexamethasone (7.5 µg/kg/day) and FWGE (1.25 g/kg/day) was even less effective (FIG. 7B). Lower doses of FWGE (1.0 g/kg/day), dexamethasone (6 µg/kg/day) and also of diclofenac (0.4 mg/kg/day) showed moderate anti-inflammatory efficacies and so did the combination of FWGE (0.5 g/kg/day) and dexamethasone (3.0 µg/kg/day). The combination of FWGE (0.5 g/kg/day) and diclofenac (0.2 mg/kg/day), however, decreased the inflammation to a minimum level (FIG. 7C).

## DISCUSSION

As far as we know, this is the first report on a wheat-derivative with experimental anti-inflammatory efficacy. In the first set of our experiments it was shown that FWGE had potent anti-inflammatory properties, which are likely to



**FIGURE 6.** (A) Anti-inflammatory efficacies of different doses of FWGE (A), diclofenac (Di), and their combinations in AA. (B) Anti-inflammatory efficacies of different doses of FWGE (A), dexamethasone (De), and their combinations in AA. C = control. \* $P < 0.05$ .



**FIGURE 7.** (A) Severe chronic inflammatory reaction in a control (solvent-treated) AA rat. The perisynovium shows severe lymphocyte infiltration (H and E,  $\times 300$ ). (B) The synovial tissue of a rat treated with higher doses of FWGE plus dexamethasone shows severe lymphocyte infiltration (H and E,  $\times 300$ ). (C) The synovial tissue of a rat treated with higher doses of FWGE plus diclofenac shows only slight edema without lymphocyte infiltration (H and E,  $\times 300$ ).

be mediated by cyclooxygenase inhibition. Arachidonic acid metabolites are important mediators of the inflammatory events in RA.<sup>16</sup> COX enzymes, also called prostaglandin (PG) endoperoxide synthases, catalyze the rate-limiting step in the conversion of arachidonic acid to PGH<sub>2</sub>, the precursor of a wide group of biologically active mediators, such as PGE<sub>2</sub>, prostacyclin, and thromboxane A<sub>2</sub>. There are two isoforms of COX gene encoded by distinct genes. The COX-1 protein is constitutively expressed in many tissues and cell types (e.g., in normal kidney and in the gastrointestinal and reproductive systems) and generates PGs for normal physiological functions. The COX-2 protein has a low basal expression level, but this isoform is induced in response to many

proinflammatory agents, as well as mitogens and tumor promoters. COX-2 is expressed in activated macrophages, monocytes, and several other cell types and has been identified in chronic inflammatory conditions *in vivo*. In this study, COX-1 and COX-2 mRNAs, derived from aortic blood at the time of the termination of experiment 1 (e.g., sacrifice of the rats by exsanguination) were investigated using real-time quantitative RT-PCR. The results show that FWGE regulates inflammation-induced COX-1 and COX-2 expression in AA. There is no explanation at present why the very low dose of the extract enhanced COX expression. Higher doses, however, showed a potent inhibitory activity and a dose-response relationship. It should be noted that the inhibition of COX-2 might also play a role in the known antitumor activity of FWGE, especially in colorectal cancer.<sup>17</sup> The effect of indomethacin on the COX-2 gene expression is striking. To our knowledge this is the first report regarding the stimulatory activity of indomethacin on the COX-2 gene. In the second set of experiments FWGE showed additive effect with diclofenac but not with dexamethasone. Using the doses of corresponding efficacy on AA, the degree of anti-inflammatory effects was also comparable in the histological grading. Since both diclofenac and dexamethasone are routinely used in the treatment of RA, the comparable anti-inflammatory properties of FWGE with these drugs in AA seems very promising, especially when the lack of toxicity of FWGE<sup>18</sup> is considered. While FWGE repeatedly showed efficacy on the noninjected paws, the result on the injected ones was not as obvious. Following the adjuvant injection in the paw, first a local inflammation developed and then this local process was associated with the general (immune-mediated) flogistic reaction. Therefore, in the injected paw, the local inflammation (mediated by histamine, serotonin, bradykinin, etc.) might disturb or mask the effect of any substance on the general immune-mediated inflammatory response. This might explain why FWGE exhibited a significant and reproducible inhibition in AA in the noninjected paw and not as clear but detectable efficacy in the injected one. So far, we have no explanation why the combined therapy with FWGE and dexamethasone was weakened compared to monotherapy with either FWGE or dexamethasone.

The pathogenetic importance of synovial T cells in RA and particularly CD4+ T lymphocytes as the perpetuators of this disease is generally accepted, and substances that cause apoptosis of activated T cells of antigen-presenting cells (APCs) may lead to a more effective and specific therapy in RA.<sup>19</sup> In this study the infiltrating lymphocytes almost entirely disappeared upon FWGE treatment. Therefore, any changes in the CD4+ subset of lymphocytes could not be measured. The lack of infiltration also points to the immune regulatory effect of this substance.<sup>20</sup> As has already been mentioned, FWGE administration improved disease outcome in RA patients.<sup>12</sup> Our experimental results also suggest that FWGE could be implemented as a suitable therapeutic tool in the treatment of RA in humans or in animals.

## REFERENCES

1. JAKAB, F., Y. SHOENFELD, A. BALOGH, *et al.* 2003. A medical nutriment has supportive value in the treatment of colorectal cancer. *Br. J. Cancer* **89**: 465–469.
2. DEMIDOV, L.V., L.V. MANZJUK, G.Y. KHARKEVITCH, *et al.* 2002. Antimetastatic effect of Avemar in high-risk melanoma patients. *Int. J. Cancer* **100**(Suppl 13): S408.
3. BARABAS, J. & Z. NEMETH. 2006. Recommendation of the Hungarian Association for Face, Mandible and Oral Surgery in the indication of supportive therapy with Avemar [in Hungarian]. *Orv. Hetil.* **147**: 1709–1711.
4. HIDVEGI, M., J. MOLDVAY, K. LAPIS, *et al.* 2003. Fermented wheat germ extract improves quality of life in lung cancer patients [in Hungarian]. *Medicus Anonymus/Pulmono* **11**(Suppl 1): 13–14.
5. GARAMI, M., D. SCHULER, M. BABOSA, *et al.* 2004. Fermented wheat germ extract reduces chemotherapy-induced febrile neutropenia in pediatric cancer patients. *J. Pediatr. Hematol. Oncol.* **26**: 631–635.
6. TELEKES, A., E. KISS-TOTH, T. NAGY, *et al.* 2005. Synergistic effect of Avemar on proinflammatory cytokine production and Ras-mediated cell activation. *Ann. N. Y. Acad. Sci.* **1051**: 515–528.
7. FAJKA-BOJA, R., M. HIDVEGI, Y. SHOENFELD, *et al.* 2002. Fermented wheat germ extract induces apoptosis and downregulation of major histocompatibility complex class I proteins in tumor T and B cell lines. *Int. J. Oncol.* **20**: 563–570.
8. COMIN-ANDUIX, B., L.G. BOROS, S. MARIN, *et al.* 2002. Fermented wheat germ extract inhibits glycolysis/pentose cycle enzymes and induces apoptosis through poly(ADP-ribose) polymerase activation in Jurkat T-cell leukemia tumor cells. *J. Biol. Chem.* **277**: 46408–46414.
9. BOROS, L.G., K. LAPIS, B. SZENDE, *et al.* 2001. Wheat germ extract decreases glucose uptake and RNA ribose formation but increases fatty acid synthesis in MIA pancreatic adenocarcinoma cells. *Pancreas* **23**:141–147.
10. SAIKO, P., M. OZSVAR-KOZMA, S. MADLENER, *et al.* 2007. Avemar, a nontoxic fermented wheat germ extract, induces apoptosis and inhibits ribonucleotide reductase in human HL-60 promyelocytic leukemia cells. *Cancer Lett.* **250**: 323–328.
11. ILLMER, C., S. MADLENER, Zs. HORVATH, *et al.* 2005. Immunologic and biochemical effects of the fermented wheat germ extract Avemar. *Exp. Biol. Med.* **230**:144–149.
12. BALINT, G., A. APATHY, M. GAAL, *et al.* 2006. Effect of Avemar – a fermented wheat germ extract – on rheumatoid arthritis. Preliminary data. *Clin. Exp. Rheumatol.* **24**: 325–328.
13. NAPARSTEK, Y., A. HERSHKO, R. ULMANSKY, *et al.* 2006. Antibodies against an HSP epitope suppress autoimmune arthritis and diabetes by modulation of cytokine regulation. 5th International Congress on Autoimmunity. Sorrento, Italy, Nov. 29 – Dec. 3.
14. CARLSON, R.P. & P.B. JACOBSON. 1998. Comparison of adjuvant and streptococcal cell wall-induced arthritis in the rat. *In In vivo Models of Inflammation*. D.W. Morgan and L.A. Marshall, Eds.: 1–50. Birkhäuser Verlag, Basel–Boston–Berlin.
15. WEICHMAN, B.M. 1989. Rat adjuvant arthritis: a model of chronic inflammation. *In J.Y. Chang & A.J. Lewis, Eds.: 363–380. Pharmacological Methods in the Control of Inflammation*. Alan R. Liss, Inc., New York.

16. SAMUELSSON, B. 1991. Arachidonic acid metabolism: role in inflammation. *Z. Rheumatol.* **50**(Suppl 1): 3–6.
17. FARKAS, E. 2005. Fermented wheat germ extract in the supportive therapy of colorectal cancer [in Hungarian]. *Orv. Hetil.* **146**: 1925–1931.
18. HEIMBACH, J.T., G. SEBESTYEN, G. SEMJEN, *et al.* 2007. Safety studies regarding a standardized extract of fermented wheat germ. *Int. J. Toxicol.* **26**: 253–259.
19. POPE, R.M. 2002. Apoptosis as a therapeutic tool in rheumatoid arthritis. *Nat. Rev. Immunol.* **2**: 527–535.
20. HIDVEGI, M., E. RASO, R. TOMOSKOZI-FARKAS, *et al.* 1999. Effect of MSC on the immune response of mice. *Immunopharmacology.* **41**: 183–186.