Lactobacillus acidophilus maintained oxidative stress from reproductive organs in collagen-induced arthritic rats

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ABSTRACT

CONTEXTS: Nonsteroidal anti-inflammatory drugs (NSAIDs) induced organ damage is a well-known fact. Previous studies suggest that *Lactobacillus* scavenge the free radicals from liver and kidney and also protect animals from arthritis. **AIMS:** Comparing protective properties of *Lactobacillus acidophilus* in reducing oxidative stress from reproductive organs developed during collagen-induced arthritis in animal model. **METHODS:** Arthritis was induced in Wistar rats. Oral administration of *L. acidophilus*, indomethacin, and distilled water were all started on the same day. Arthritis scores were calculated for each group. Oxidative stress parameters were estimated in testis and ovary homogenates. Histopathology of ovary and testis was also performed. **RESULTS AND CONCLUSION:** *L. acidophilus* decreased arthritis score ($P < 0.001$) as well as maintained normal histology of reproductive organs. *L. acidophilus* maintained oxidative stress parameters from ovaries and testis ($P < 0.001$). These results provide strong evidence that NSAIDs increase oxidative stress in reproductive organs while *L. acidophilus* not only scavenges free radicals from reproductive organs but also protects rats from arthritis symptoms.

KEY WORDS: Collagen-induced arthritis, cyclooxygenase, *Lactobacillus acidophilus*, ovary, oxidative stress, probiotics, testis

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as an important therapeutic regime to suppress pain and inflammation. NSAIDs have analgesic, antipyretic, and anti-inflammatory properties.[1,2] Though exact mechanism of action of NSAIDs is not understood yet, inhibition of cyclooxygenase enzyme (both COX 1 and COX 2) that converts arachidonic acid to prostaglandins is likely to be the reason behind their action. These drugs lessen symptoms, but they have side effects. They damage liver, kidney, nervous system, gastrointestinal tract, and also blood by increasing toxicity.[3-6]

NSAIDs interfere with prostaglandins synthesis and may be responsible for delay in release of mature ovum (i.e., luteinized unruptured follicle).[7] They hinder ovulation by inhibiting COX 1 and COX 2 enzymes.[8] This results in hindrance or failure of ovulation.[9] In a study, the targeted disruption of COX 2 in mice produced multiple failures in female reproductive processes including ovulation, fertilization, implantation, and decidualization.[10] NSAIDs therapy caused reduction in seminal volume, total number of spermatozoa, percentages of motile, viable and morphologically normal cells, and fructose levels in males.[11] Similarly, prostaglandins E and F play an important role in sperm metabolism and its function.

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Oxidative stress has been associated with the pathogenesis of many diseases including cancer, diabetes mellitus, rheumatoid arthritis, Parkinson's disease, and systemic lupus erythematosus.[12] Drug-induced oxidative stress is linked with toxicity of various tissues and organ systems. Sometimes drug uptake may generate reactive intermediates that can scavenge molecular oxygen level directly to generate reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS play an important role in improper functioning of reproductive system and cause sterility.[13] These reactive oxygen/nitrogen intermediates with prostanoids, leukotrienes, and proteases facilitate inflammation and may lead to tissue destruction.

Herbal formulations or herb-based drugs are known to be effective against many diseases and ailments and have no side effects.[14,15] Probiotics are consumed as food since long time back. However, now their use has been changed to dietary feed supplements. They are immune-enhancer.[16,17] Previous studies by our group suggest that *Lactobacillus casei* and *Lactobacillus acidophilus* possess anti-arthritic activity and free-radical scavenging properties in the liver and kidney homogenates.[18] As a part of ongoing research, antioxidant properties of *L. acidophilus* were investigated from reproductive organs by using collagen-induced arthritis (CIA) model. Results of *L. acidophilus* were compared with reference drug indomethacin (NSAIDs).

METHODS

Bacteria

L. acidophilus (ATCC 314) was used in this study. De Mann Rogosa Agar (MRS) was used to streak lyophilized culture at 37°C in anaerobic condition.

Animals and experimental procedures

The study was conducted on 24 male and female Wistar rats (7 weeks old) weighing 230 g. Male and female rats were individually housed with a temperature range of 25 ± 3°C. During the experiment, all guidelines of Institute's Ethical Committee were strictly followed.

Adjuvant, drug, and reagents

Bovine tracheal cartilage Type II collagen was purchased from Roche Diagnostics GmbH and incomplete Freund's adjuvant from Sigma. Standard anti-arthritic drug indomethacin was purchased from Recon.

Oral administration of *Lactobacillus acidophilus*, drug, and vehicle

All the animals were randomly distributed into control and treated test-groups. Effects of *L. acidophilus* and reference drug indomethacin were investigated by prophylactic protocol. Treatments were given from the day 1, until end of the experiment (28th day). Grouping of animals were as follows:

Grouping of female rats (6 each):

- Group I: Negative control, i.e., no arthritis and no treatment
- Group II: Positive control, i.e., arthritis induced and 0.5 ml of distilled water
- Group III: 2 × 10^8 colony-forming unit (CFU)/ml of *L. acidophilus* suspended in 0.5 ml of distilled water
- Group IV: Standard drug "Indomethacin" at 10 mg/kg of body weight.

Grouping of male rats (6 in each group):

- Group I: Negative control, i.e., no arthritis and no treatment
- Group II: Positive control, i.e., arthritis and 0.5 ml of distilled water
- Group III: Arthritis induced and 2 × 10^8 CFU/ml of *L. acidophilus* suspended in 0.5 ml of distilled water
- Group IV: Arthritis-induced standard drug "Indomethacin" at 10 mg/kg of body weight.

Induction of arthritis

CIA was given as suggested by Remmers *et al*.[19]

Arthritis score

Clinical symptoms of arthritis were evaluated on a four-point scale by visual appearance at the end of each week**.** Scores were calculated on a scale of 0–4 point scale: 0 = no swelling or erythema, 1 = slight swelling and/or erythema, 2 = low to moderate edema, 3 = pronounced edema with limited joint usage, and 4 = excess edema with joint rigidity. Total score for each animal was then calculated and used as an articular index.

Collection of tissues, assessment of oxidative stress, and histopathology

On the 29th day, all the animals were sacrificed. Ovaries and testis were dissected and kept in 10% formalin for 24 h for histopathology. Supernatant fluid from testis and ovary was used for estimating various antioxidant enzymes. Levels of reduced glutathione (GSH),[20] catalase,[21] superoxide dismutase (SOD),[22] lipid-peroxidation,[23] and GSH peroxidase (GPx)[24] were estimated.

Data analysis

Results obtained from different groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. Data were considered statistically significant if $P < 0.001$.

RESULTS

Results suggested that *L. acidophilus* elicited significant anti-arthritic properties and maintained reproductive organs in CIA in both female and male experimental rats.

Arthritis score

Results of arthritis score have been shown in Figure 1a and b. For male rats, Group I animals were normal, and they were showing arthritis score of 0 from the beginning to the end of experimental protocol (i.e., on the 28th day). No changes were observed in Group I animals. Groups II, III, and IV were showing redness and swelling at the end of the 1st week. The highest arthritis score was observed for Group II animals (7.62 ± 0.12), which was significant to all other groups. Group III and IV were showing arthritis score of 6.37 ± 0.07 and 6.52 ± 0.02, respectively. Group II animals were showing an increase in arthritis score of 12.35 ± 0.05 and 14.37 ± 0.07 on 21st and the 28th day. *L. acidophilus* treatment significantly lowered arthritis score in the 3rd and 4th week, which was found to be significant when compared to Group II except on the 14th day. The standard drug also decreased the arthritis score in Group IV animals [Figure 1a].

Female rats were also showing similar results as of male rats [Figure 1b]. Arthritis score was the highest in Group II animals on the 28th day, whereas *L. acidophilus* treatment significantly decreased arthritis score in Group III animals on the 28th day. Standard drug indomethacin (Group IV) has also shown a considerable decrease in arthritis score on the day 28.

Oxidative stress parameters

In ovary homogenates, levels of catalase and lipid peroxidation were significantly decreased by *L. acidophilus* treatment compared to positive control which was significant when compared to other groups [Figure 2]. Furthermore, *L. acidophilus* treatment increased GSH, GPx, and SOD in Group III animals. The drug was also showing comparable results.

In testis homogenates, oral administration of *L. acidophilus* decreased catalase and lipid peroxidation levels [Figure 3]. Furthermore, decreased level of GSH, GPx, and SOD was rescued by feeding male rats with *L. acidophilus*.

Histopathology

Major histological changes were observed in Group II and Group IV when compared with Groups I and III

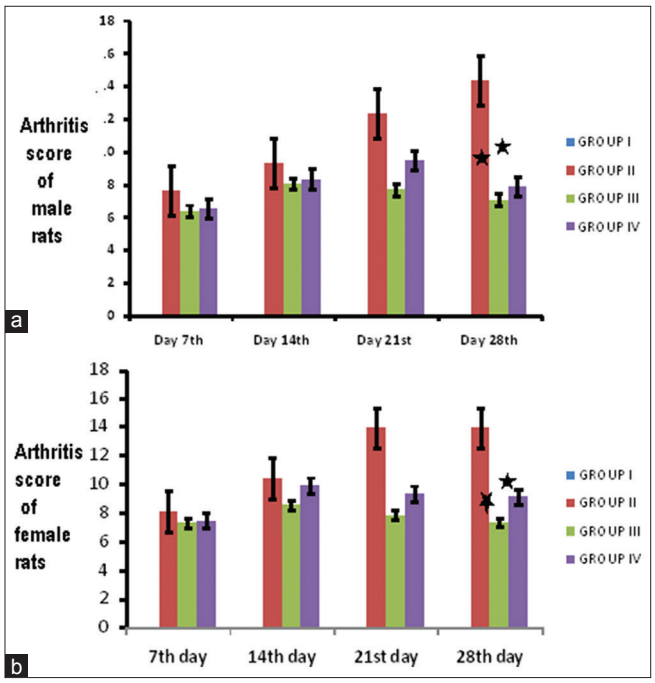

Figure 1: Evaluated arthritis score in female (F) and male (M) rats (mean ± standard error of the mean, 6 rats/group). Group I: No arthritis induction and no oral treatment; Group II: Arthritis induction and distilled water; Group III: Arthritis induction and orally administered *Lactobacillus acidophilus*; Group IV: Arthritis induction and standard drug indomethacin administered orally. Data are expressed as mean ± standard error of six rats per group. *P < 0.001 Group II versus all other treated group

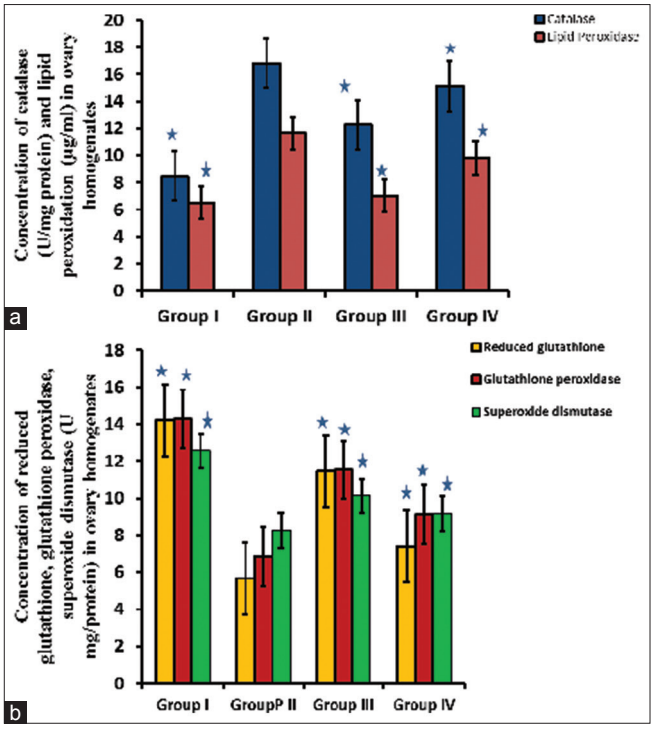

Figure 2: Evaluated oxidative stress parameters in ovary homogenates (mean ± standard error of the mean, 6 rats/group). Group I: No arthritis induction and no oral treatment; Group II: Arthritis induction and distilled water; Group III: Arthritis induction and orally administered *Lactobacillus acidophilus*; Group IV: Arthritis induction and standard drug indomethacin administered orally. *P < 0.001 Group II versus all other treated group

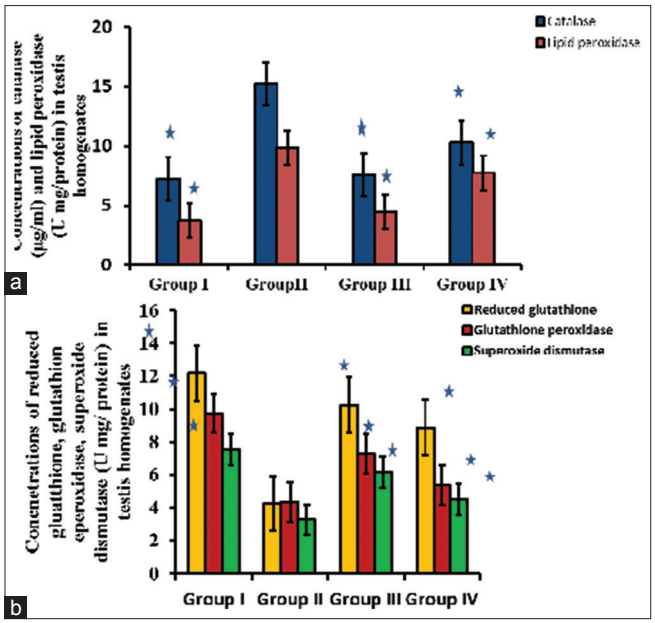

Figure 3: Evaluated oxidative stress parameters in testis homogenates (mean ± standard error of the mean, 6 rats/group). Group I: No arthritis induction and no oral treatment; Group II: Arthritis induction and distilled water; Group III: Arthritis induction and orally administered *Lactobacillus acidophilus*; Group IV: Arthritis induction and standard drug indomethacin administered orally. *P < 0.001 Group II versus all other treated group

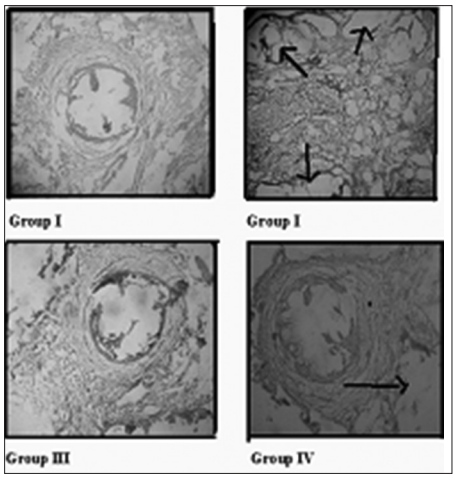

Figure 4: Histopathology of ovary in female rats. Pictures are representative of distinct six rats per group. Arrows indicate abnormality and irregularity in normal histology. Group I: No arthritis induction and no oral treatment; Group II: Arthritis induction and distilled water; Group III: Arthritis induction and orally administered *Lactobacillus acidophilus*; Group IV: Arthritis induction and standard drug indomethacin administered orally

animals (both female and male rats). Lysed ova were seen in Group II and Group IV animals. Damaged and degenerative changes were relatively less in Group III while compared with Groups II and IV animals [Figure 4].

Examination of testis revealed that no histological changes were observed in Group I animals while Group II rats were showing large numbers of seminiferous tubules in comparison to all other groups. Large vacuoles and necrotic areas were seen in Group II and Group IV animals. Less destruction was observed in Group III when compared to Group IV animals [Figure 5].

DISCUSSION

Findings of this study clearly suggest that *L. acidophilus* shows outstanding protective properties in ovaries and testis homogenates as evaluated by antioxidative efficiency. They also confirm our previous finding that *L. acidophilus* possesses anti-arthritic properties.[25] In this study, *L. acidophilus* suppressed joint inflammation and synovitis. It was effective in preventing systemic spread and ultimately destruction of joints as validated by arthritis scores. Decrease in arthritis score in *L. acidophilus* administered male and female rats is suggestive of anti-arthritic activity of *L. acidophilus.*

NSAIDs inhibit coxygenase enzyme. Coxygenase is responsible for prostaglandins synthesis which plays an important role in physiological functioning.[15] COX 1 is a useful "housekeeper" isoform and is involved in regulation of cellular and metabolic activities while COX 2 is "inducible" isoform, which is responsible for activating pro-inflammatory stimulus. COX 2 originates in the brain, kidneys, osteoblasts, ovaries, and testis.[26] NSAIDs alter several intracellular signaling pathways such as GMP-dependent kinase,[27] activates stress-related kinases such as c-Jun N-terminal kinases and mitogen-activated protein kinases p38 while inactivating extracellular signal-regulated kinases.[28] This was the basis of evaluating oxidative stress associated with reproductive organs in this study. Previous studies by our group suggest that *L. casei* and *L. acidophilus* maintained antioxidant status in liver and kidney homogenates.[18] Therefore, in this study, free radical induced factors were evaluated in reproductive organs and compared with the reference drug.

L. acidophilus treatment significantly decreased catalase level in Group III in both male and female rats. Anti-inflammatory drug was showing higher concentration of catalase in both male and female rats. Malonaldehyde is degradation product of lipid peroxidation. Lipid peroxidation level was found to be highest in Group II animals for both male and female rats while *L. acidophilus* fed group (i.e. Group III) were showing less concentration of malonaldehyde.

Levels of GPx are of critical importance as it is natural antioxidant. GPx level was maintained to be near to normal by oral administration of *L. acidophilus*. Group III (*L. acidophilus* fed) shown an increased GPx level for male and female rats, respectively, which were also found to be

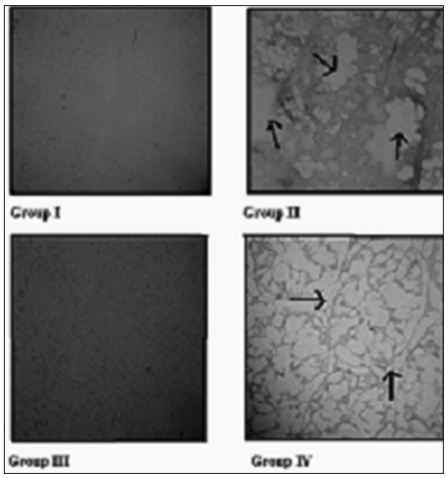

Figure 5: Histopathology of testis in male rats. Pictures are representative of distinct six rats per group. Arrows indicate abnormality and irregularity in normal histology. Group I: No arthritis induction and no oral treatment; Group II: Arthritis induction and distilled water; Group III: Arthritis induction and orally administered *Lactobacillus acidophilus*; Group IV: Arthritis induction and standard drug indomethacin administered orally

significant when compared to Group II (positive control) and Group IV (drug). GSH level in both male and female rats was increased by *L. acidophilus* administration. Similar results were also found in case of SOD.

Histopathology also supported our findings. Oral administration of *L. acidophilus* decreased testicular and ovarian damage. Group I showed normal cell arrangement when compared to Group II animals for both male and female rats. Indomethacin treatment showed irregular arrangement of cells and reduced cell size in Group III animals. It can be revealed that *L. acidophilus* treatment scavenged free radicals that were generated and hence protected ovary and testis from oxidative damage.

Administration of *Lactobacillus* and its protective properties were shown in many studies.[29,30] Moreover, *L. casei* and *L. acidophilus* decreased oxidative stress from synovial fluid in CIA model.[31]

ROS plays wide role in normal cell signaling and maintaining homeostasis.[32] Free radicals cause damage to all biomolecules and obstruct their vital function. ROS and oxidative stress are now believed to be leading underlying pathology linking varicocele with male infertility.[33-35]

Organ toxicity is considered as the most common cause and severe characteristics of NSAIDs. It is still not clear whether these side effects are caused by "metabolic idiosyncrasy" or by "immunological idiosyncrasy". Specific COX 2 inhibitors which reduce inflammation and pain without inhibiting the protective prostaglandins of important organs are important area of research. *Lactobacillus* having potent anti-inflammatory and anti-arthritic properties also protects reproductive organs from inflammatory reaction induced in arthritis. Possible mechanism of action of *L. acidophilus* behind the antioxidant status in ovaries and testis is a question unanswered.

CONCLUSION

L. acidophilus possesses anti-arthritic, antioxidant, and protective properties in both male and female Wistar rats. It has maintained oxidative stress in reproductive organs. Histopathological analysis of testis and ovaries also revealed that *L. acidophilus* provided protection from damage. Our study also validated the fact that *L. acidophilus* can be a promising candidate against arthritis. In addition, since NSAIDs show deleterious effects on important organs including testis and ovaries, such new treatment options (i.e. *L. acidophilus*) may be superior in many pathophysiological aspects.

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Conflicts of interest
There are no conflicts of interest.

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