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Original Research Article

Evaluation of anti-aging effects of swimming exercise on inflammatory parameters in D-galactose-treated Wistar albino male rats

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ABSTRACT

Background: The present study was undertaken to investigate the anti-ageing effect of swimming exercise on inflammatory parameters in D-galactose-induced Wistar albino male rats.
Methods: Wistar albino rats were divided into four groups, namely, normal control, D-galactose-treated group (positive control), normal control with swimming exercise, and D-galactose-treated rats with swimming exercise. The experiment was conducted for 42 days. D-galactose was administered as a compound to induce ageing-like changes, and swimming exercise was used as a non-pharmacological treatment. Plasma was separated from blood samples on the 43rd day for estimation of inflammatory markers, interleukin-6 (IL-6) and c-reactive protein (CRP) taken from the tail vein.
Results: The plasma levels of IL-6 and CRP were significantly higher in the D-galactose-treated rats than in the normal control rats, suggesting an enhanced inflammatory response. The swimming exercise significantly reduced IL-6 and CRP levels in D-galactose-treated rats compared with the positive control group.
Conclusions: Swimming exercise was found to have a significant anti-inflammatory and anti-aging effect on Wistar albino male rats treated with D-galactose, as the plasma levels of IL-6 and CRP were reduced. Therefore, swimming exercise could be regarded as a useful non-pharmacological method to prevent inflammatory changes associated with aging.

Keywords: Aging, C-reactive protein, D-galactose, Inflammation, Interleukin-6, Swimming exercise

INTRODUCTION

Aging is a natural, ongoing, biological process accompanied by progressive loss of cellular, biochemical and physiological functions. It's linked with oxidative stress, mitochondrial dysfunction, cellular aging, and decreased repair mechanisms.[1]

One of the important mechanisms for ageing is chronic low-grade inflammation. The continuous inflammatory state is often referred to as the inflammaging.[1]

Inflammaging is linked with higher levels of inflammatory markers like c-reactive protein and interleukin-6. These markers are usually increased during ageing and age-related diseases.[3]

Interleukin-6 is a pro-inflammatory cytokine that has a major role in systemic inflammatory responses. Aging is related to high IL-6 levels and a loss of function.[4]

C-reactive protein (CRP), an acute-phase protein, is a commonly used systemic inflammatory marker. An elevated CRP is a sign of inflammation.[5]

The D-galactose-induced aging is a widely used experimental model of aging in animals. Chronic D-galactose administration causes oxidative stress, inflammation and cell damage.[6]

Thus, D-galactose-treated Wistar albino rats are ideal for testing anti-aging therapeutics.[7]

Regular exercise is an effective non-pharmacological intervention for promoting health and minimising functional decline associated with aging.[8]

In experimental animal studies, swimming exercise is usually employed as a controlled aerobic exercise. It enhances physiological adaptation, and it can decrease inflammatory responses.[9]

However, the present study was carried out to assess the anti-aging activity of swimming exercise on inflammatory parameters in D-galactose induced aging Wistar albino male rats. The study mainly focused on plasma IL-6 and CRP levels to assess the anti-inflammatory role of swimming exercise.

METHODS

Chemicals

D-galactose was purchased from Sisco Research Laboratories Pvt. Ltd., India. Interleukin-6 (IL-6) and c-reactive protein (CRP) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Sigma-Aldrich Pvt. Ltd., India. All chemicals used in the study were of analytical grade.

Experimental animals

Male Wistar albino rats (250-300 gm) were used for the study. Animals were kept in standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature and humidity. Pellet food and water were given freely during the whole experimental period.

Ethical approval

This study protocol was approved by the Institutional Animal Ethics Committee for K. S. Hegde Medical Academy (Approval No. 117/1999). The experiment was performed in accordance with the guidelines of CPCSEA for the welfare and management of laboratory animals.

The study was conducted from August 2018 to April 2019. The experimental intervention was carried out over a period of 42 days. Animals were randomly grouped into four groups of six rats each.

Design

It was an experimental design. Animals were divided into four groups: group I: normal control, group II: positive control (D-galactose-treated group), group III: normal control + swimming exercise, group IV: swimming exercise + D-galactose-treated group.

Oral administration of D-galactose at a dose of 100 mg/kg/day for 42 days induced aging-like changes.

Swimming exercise protocol

The rats in the swimming exercise groups underwent swimming exercise in a cylindrical swimming tank filled with room temperature water. Swimming exercise was performed every day for the experiment period. Caution was taken to prevent unnecessary stress and fatigue in the exercise protocol.[11]

Blood sample collection

Blood sampling was done in rats on day 43 from the tail vein. Plasma was separated and analysed for estimation of inflammatory parameters: IL-6 and CRP.

Estimation of IL-6

IL-6 levels in plasma were estimated using an enzyme-linked immunosorbent assay (ELISA) kit as per the manual provided. Absorbance was determined by an ELISA reader, and the concentration of IL-6 was determined by the standard calibration curve.[12]

Estimation of CRP

Plasma c-reactive protein (CRP) level was determined using a commercially available c-reactive protein enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Absorbance was read by an ELISA reader, and the concentration of CRP was determined by reading the absorbance on the standard curve.[12]

Statistical analysis

Data are presented as mean±SD. Data were analysed using GraphPad Prism version 5.0. One-way analysis of variance and Tukey post hoc were performed to compare the comparison between groups. Significant p values were considered to be <0.05.

RESULTS

The effect on plasma IL-6 levels

Plasma interleukin-6 (IL-6) level was significantly elevated in the D-galactose-treated positive control group compared to that of the normal control group (Figure 1), indicating the rise in inflammatory response. The IL-6 level

was significantly decreased compared to the positive control group in D-galactose-treated rats with swimming exercise. The identification of this finding indicates that the swimming exercise had a beneficial effect on decreasing the inflammatory changes caused by the D-galactose.

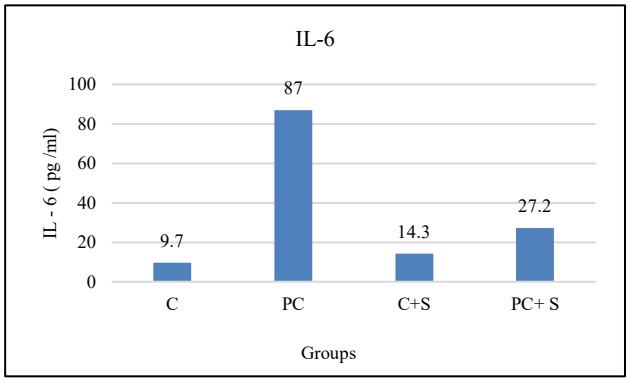

Figure 1: The effect of swimming exercise on plasma levels of interleukin-6 in D-galactose-treated Wistar albino male rats.

Data are presented as mean±SD (n=6).

The effect on plasma CRP levels

Systemic inflammation was observed in the D-galactose-treated positive control group, as demonstrated by the increased level of plasma c-reactive protein (CRP) compared with the normal control group (Figure 2). The level of CRP was significantly decreased in D-galactose-treated rats compared to the positive control group after swimming exercise. Swimming exercise showed an anti-inflammatory effect by lowering the level of CRP.

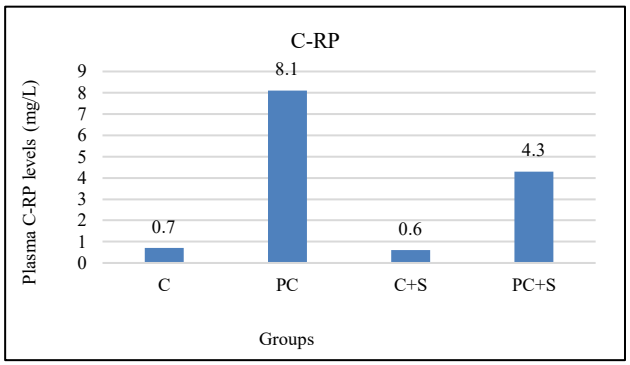

Figure 2: The effect of the swimming exercise on plasma C-reactive protein in the D-galactose-treated Wistar albino male rats.

Data are presented as mean±SD (n=6).

Overall effect of swimming exercise

Overall, it was found that administration of D-galactose significantlyx elevated plasma levels of IL-6 and CRP, indicating inflammatory changes in aging. IL-6 and CRP levels were markedly reduced by swimming exercise in D-galactose-treated rats. This finding means that swimming exercise has anti-inflammatory and anti-aging effects on D-galactose-induced Wistar albino male rats.

DISCUSSION

The anti-inflammatory effect of the swimming exercise was studied in D-galactose-treated Wistar albino male rats. The D-galactose-treated positive control group showed increased plasma IL-6 and CRP levels, indicating the development of systemic inflammatory changes. This discovery helps substantiate the theory of inflammation as a major factor in the biological changes of aging.[13]

IL-6 is a major pro-inflammatory cytokine that is associated with ageing. Raised levels of IL-6 have been associated with chronic inflammation and age-related loss of function. In the current study, the elevated levels of IL-6 in D-galactose-treated rats indicate activation of inflammatory pathways.[5]

CRP is an acute-phase protein, primarily synthesised in response to inflammatory cytokines. Elevated CRP levels indicate systemic inflammation and are often seen in age-related inflammatory conditions. We also observed an increase in CRP in positive controls, which further supports the inflammatory effect of the D-galactose treatment.[4]

The swimming exercise group rats showed a significant decrease in IL-6 protein levels compared with the positive control rats that were treated with D-galactose. Exercising in the form of regular swimming might suppress the secretion of inflammatory cytokines, and the inflammatory reaction occurring with aging.[14]

The rats that received the exercise (swim) also showed a significant reduction in the level of CRP. This implies that swimming exercise can have an anti-inflammatory effect and enhance the inflammatory status in experimental aging.[15]

The anti-inflammatory properties of exercise can be explained using repeated physiological adaptation, modulation of cytokine release and optimisation of cytokine control and regulation. Regular moderate exercise has been reported to have beneficial anti-inflammatory effects and may be protective against chronic inflammatory diseases.[16]

The present study has certain limitations. The sample size was relatively small, and only two inflammatory biomarkers were assessed. Other biomarkers of oxidative stress, cellular senescence, and molecular aging pathways were not evaluated. In addition, the findings are limited to an experimental animal model and may not be directly extrapolated to humans. Further studies involving larger sample sizes and additional molecular markers are required to better understand the anti-aging mechanisms of swimming exercise.

CONCLUSION

In the present study, swimming exercise was seen to cause a significant anti-inflammatory effect in the D-galactose-treated Wistar albino male rats. Plasma IL-6 and CRP levels were elevated after administration of D-galactose, suggesting inflammatory changes associated with aging. These inflammatory markers were significantly reduced by the swimming exercise, indicating the anti-inflammatory effect of the exercise.

Therefore, swimming exercise can be recommended as a non-pharmacological treatment to decrease the inflammatory changes of ageing. Further studies are needed to clarify its molecular mechanisms and to confirm its use in diseases related to aging.

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