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Immobilization induced degeneration in intervertebral discs of rats and protective effects of omega 3 fatty acids and CoQ10

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SUMMARY

The aim of the study was to determine the effects of immobilization on the radiological indices of rats' intervertebral disc (IVD) and observe the protective effects of Omega 3 fatty acids and Co-enzyme Q 10 (CoQ10). The study comprised 40 *Sprague-Dawley* rats weighing 250-300g. After random selection, rats were divided into four groups having 10 animals in each. Group A rats, on standard lab diet, served as control group. Group B rats were disc immobilized by using an Illizarov-type apparatus which was applied for 60 days. Group C and D rats after disc immobilization were administrated with Omega 3 fatty acids (260mg/kg/day) (Abdou and Hassan, 2014) and CoQ10 (150mg/kg/day) (Kwong et al., 2002) through oral gavage respectively. Radiographs of rat tails were taken at the start and end of experiment to measure disc height index (DHI) and disc wedge index (DWI).

On radiological examination, mean DHI of group A was measured as 0.0581 ± 0.004 while of Group B was 0.0324 ± 0.008 which was significantly lower as compared to group A (*p*-value <0.001). Whereas in groups C and D, mean DHI were 0.0552 ± 0.003 and 0.0563 ± 0.003 respectively. Similarly mean DWI of group A was calculated as 1.086 ± 0.020 and for Group B it was 1.628 ± 0.355 which was significantly increased than group A (p value < 0.001). Mean DWI of group C and D were calculated as 1.147 ± 0.038 and 1.188 ± 0.023 respectively showing improvement after administration of Omega 3 and CoQ10.

In this study, radiological changes induced by immobilization in IVDs of the experimental rats and its reversal by omega 3 and CoQ10 were proven. Omega 3 and CoQ10 administration minimized immobilization induced degeneration of IVDs.

Key words: Intervertebral disc degeneration – Immobilization – Disc height index – Disc wedge index

INTRODUCTION

Low backache is one of the debilitating general health problems which affect millions of people and their work around the world. It is among the most common conditions requiring medical care and enormous annual medical costs causing significant loss of productivity and work output (Katz, 2006). Intervertebral disc degeneration is presently documented as one of the major causes of backache. The modern sedentary and inactive lifestyle leads to reduced physical activity. This hypomobility causes increase in weight and obesity, further impeding work efficiency and activity level. Genetics, reduced physical activity, overweight and obesity are all strong risk factors for intervertebral disc (IVD) degeneration (Samartzis et al., 2012).

IVD degeneration is a process which is radiologically characterized by loss of disc height. This process begins with breakdown of extracellular matrix in the nucleus pulposus (NP) of the IVD, resulting in reduced disc height. This is followed by increased disorganization of lamellae in the annulus fibrosus (AF), and eventually fissures and in growth of nerves and blood vessels

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(Freemont, 2009). Croxford and Yamamura studied that there is significant inflammatory response in the pathogenesis of IVD degeneration. There is an up-regulation of pro-inflammatory factors that lead towards the extracellular matrix degeneration with subsequent breakdown of its components (Croxford and Yamamura, 2005). Evidence of oxidative stress also exists in degenerated IVDs. According to Johnson, oxidative damage in IVD could originate from reactive oxygen species, leading to neo vascularization as a result of annular and endplate fissures (Johnson et al., 2001).

Scientifically supported nutritional and medical evidence have allowed nutraceuticals to come into view as being potentially effective (Dillard and German, 2000). One of the popularly used nutraceuticals is Omega 3 fatty acids, known as essential to normal growth and health, not synthesized in our body. Omega 3 fatty acids are able to inhibit a number of aspects of inflammation and give rise to anti-inflammatory and inflammation resolving resolvins and protectins. CoQ10 supplementation has shown the beneficial effects in treating various stress conditions, such as neurodegenerative diseases, congestive heart failure, ageing or cancer (Hiebert et al., 2012). As oxidative stress leads to degenerated IVDs, so the potent antioxidant property of CoQ10 can present a strong defensive mechanism against oxidative damage.

MATERIALS AND METHODS

The experiment was conducted at the Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from April to May 2015. 40 adult rats (*Sprague-Dawley* strain) weighing 250-300 grams were used for the study. They were kept in the NIH animal house. All rats received the normal animal house diet. The food and water were available ad libitum. Animals were randomly selected and divided into four groups. Animals in Group A served as control and were numbered from A1-A10. The experimental Group B, in which rats tails were immobilized using an Illazrov type apparatus (Fig. 1). Animals were anesthetized by intraperitoneal injections of 10 mg/kg xylazine and 50 mg/kg ketamine. The middle of the 8^{th} and 10^{th} coccygeal vertebrae were located by palpation and tagged with metallic markers. Before procedure, radiographs were obtained to confirm the location of these tags. Afterwards, two crossed 0.7 mm stainless steel wires were inserted percutaneously through the tagged locations on each of the two vertebrae. The wires were then attached to 35 mm diameter aluminum rings with the glue. Finally four stainless steel rods, longitudinally were passed through four holes distributed around the rings creating an Illazrov-type apparatus (James et al., 1999). In group C, the rats discs were immobilized using an Illazrov apparatus and

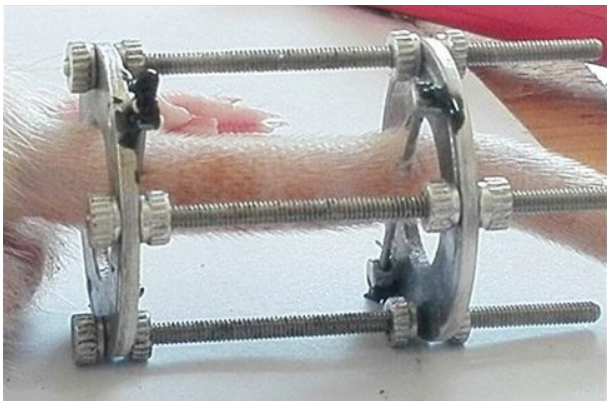

Fig 1. An Illazrov-type apparatus for rat tail segment immobilization.

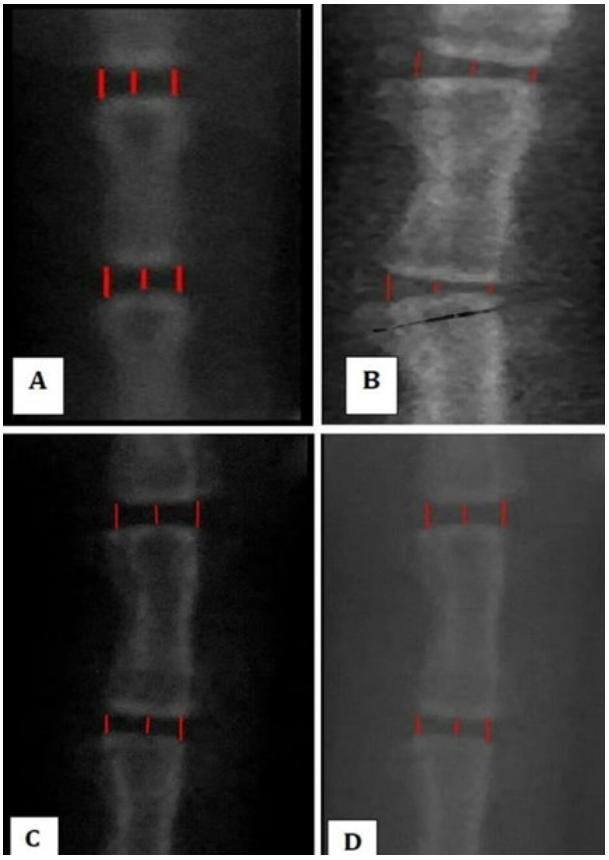

Fig 2. Radiographs of rat tail demonstrating the disc height at the end of experimental period. **A)** Control group. **B)** Disc immobilized group. **C)** Disc immobilized and omega 3 administered group. **D)** Disc immobilized and coq 10 administered group.

were administered Omega 3 fatty acids at a dose of 260 mg/kg/day through oral gavage for a period of 60 days. Intervertebral discs of rats in group D were also immobilized by an Illazrov type apparatus and were given CoQ10 (150mg/kg/day) by oral gavage for 60 days.

Image J was used for calculation of disc height index (DHI) and disc wedge index (DWI). Radiographic assessment of the disc height was performed at the start and end of experiment (Issay et al., 2013). Disc height index (DHI) and disc wedge index (DWI) were calculated

based on sagittal IVD and vertebrae height measurements (Masuda et al., 2005). Intervertebral disc height was taken at three points i-e anterior, middle and posterior, and their mean was taken as disc height.

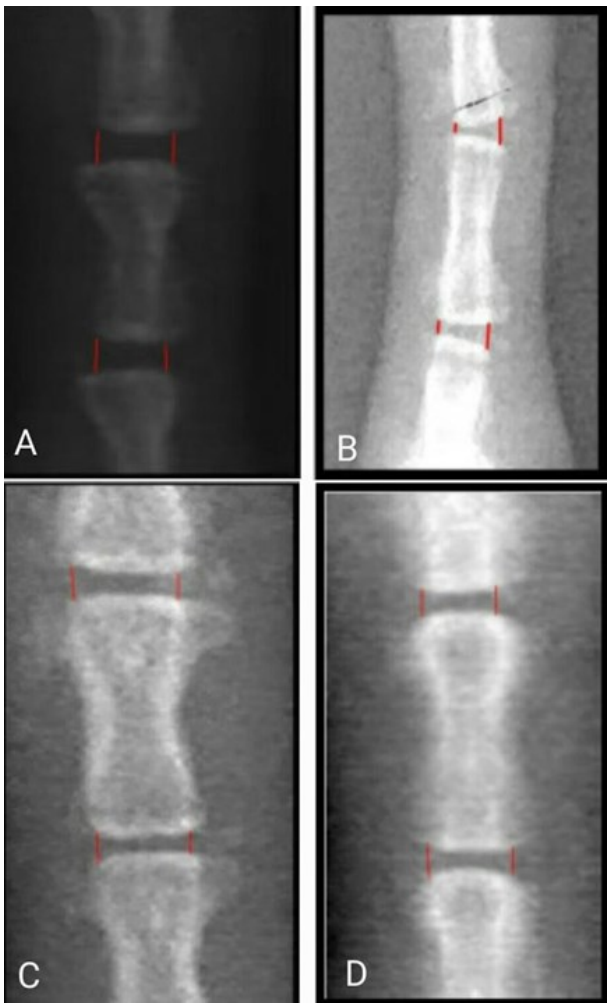

Fig 3. Radiographs of rat tail demonstrating the anterior and posterior disc heights at the end of experimental period. **A)** Control group. **B)** Disc immobilized group. **C)** Disc immobilized and omega 3 administered group. **D)** Disc immobilized and coq 10 administered group.

The DHI reflects the disc height relative to the vertebrae.

DHI= 2×IVD height/ (C8+C9)

The DWI correlates to the IVD shape.

DWI =disc height anterior/disc height posterior

SPSS 21 was used for statistical analysis. A *p*-value <0.05 was considered significant. ANOVA test was applied for intergroup comparison of quantitative (DHI and DWI) variables followed by Post Hoc Turkeys' Test. Chi square test was applied for the intergroup comparison.

RESULTS

Examination of radiographs of the control group (group A) revealed normal disc (Fig. 2A). The disc height was measured and its mean value was 0.0581 ± 0.004 (Table 1). Radiographs of the experimental Group B showed reduced disc heights (Fig. 2B). Mean DHI value of group B was 0.0324 ± 0.008 (Table 1). It was significantly lower as compared to control group A (*p*-value <0.001). Whereas in experimental groups C and D, mean values of DHI were 0.0552 ± 0.003 and 0.0563 ± 0.003 respectively (Table 1, Figs. 2C and 2D).

On intergroup comparison, DHI in Group B was reduced and was found statistically significant as compared to control group A (*p*-value < 0.001) and experimental group C (*p*-value < 0.001) and D (*p*-value <0.001) respectively **(**Table 1).

Radiological sections of Group C showed that the DHI was slightly reduced and this reduction in DHI was not found to be significant when compared to control group A (*p*-value=0.653) and group D (*p*-value=0.971). However, there was statistically significant difference between group C and group B (*p*-value=0.000) (Table 1). Similarly mean DHI value of group D showed minor difference from group A (*p*-value=0.887) and experimental group C (*p*-value=0.971), while it was statistically significant when compared to disc immobilized group B (*p*-value=0.000) (Table 1).

DWI was calculated in control group A and its mean value was 1.086 ± 0.020 (Fig. 3A). In experimental Group B mean DWI was 1.628 ±

Table 1. Comparison of quantitative radiological parameters in control group A, experimental group B, group C and group D.

Parameters	Groups	Mean±SD	Comparison of p-values					
			Group A/B	Group A/C	Group A/D	Group B/C	Group B/D	Group C/D
Disc Height Index(DHI)	A	0.058±0.004	0.000	0.653	0.887	0.000	0.000	0.971
	B	0.032±0.008						
	C	0.055±0.003						
	D	0.056±0.003						
Disc Wedge Index(DWI)	A	1.086±0.020	0.000	0.872	0.587	0.000	0.000	0.956
	B	1.628±0.355						
	C	1.147±0.038						
	D	1.188±0.023						

0.355, which was significantly more than control group A (Fig. 3B, Table 1). Whereas in experimental groups C and D, mean DWI were 1.147 ± 0.038 and 1.188 ± 0.023 respectively (Figs. 3C and 3D, Table 1). The values were slightly increased but were close to the control group A but were significantly lower than group B.

DWI in experimental group D had minimal increase which was not found to be statistically significant as compared to control group A (*p*-value=0.587) and experimental group C (*p*-value=0.956), while it was statistically significant when compared to disc immobilized group B (*p*-value=0.000) (Table 1).

On intergroup comparison it was observed that DWI in experimental Group B was increased as compared to control group A (*p*-value=0.000) (Table 1). While in group C slight increase in DWI was seen which was not found significant as compared to group A (*p*-value=0.872) and group D (*p*-value=0.956) but was found statistically significant as compared to group B (*p*-value=0.000) (Table 1).

DISCUSSION

Disc height was assessed radiologically by measuring DHI and DWI. In this study the mean DHI in group B was significantly reduced when compared with control group A and experimental omega administrated group C and CoQ10 administered group D. This increase in DHI of group B (0.324 ± 0.008) was statistically significant ($p < 0.001$) when it was compared with control group A and experimental group C and D. The results of the current investigations were in agreement with the findings of James who proved that immobilization causes reduction in disc height and disc height index (James et al., 1999). IVD degeneration is a chronic inflammation which is associated with catabolic shift of IVD metabolism, leading to increased cell death and loss in glycosaminoglycan (GAG) content. These tissue changes lead to reduced hydration, increased stiffness of IVD and loss of overall IVD height (Hughes et al., 2012). IVD degeneration leads to rise in pro-inflammatory cytokines, leading to extracellular matrix degradation, which can be targeted by omega 3 to halt this process (Makarand and Irving, 2014). The mean DHI in the omega 3 administered group C was also higher as compared to the group B. This is attributed to the fact that omega 3 fatty acids when consumed in sufficient quantities, results in decreased pro-inflammatory cytokines and leukocyte chemotaxis. Maintenance of disc height in omega 3 administered group is also in agreement with findings of Calder who proved that omega 3 supplementation results in decreased production of cytokines, leukotriene B4 and PGE2 by inflammatory cells (Calder, 2006). The mean DHI in the CoQ10 administrated group D was also higher as compared to the group B. This is because CoQ10 have an anti-oxidant action and it may be recommended for the prevention of pathologies associated with oxidative stress. According to Luigi oxidative stress exists in degenerated discs, by decreasing matrix synthesis and increasing gene expression of catabolic factors. Major source of reactive oxygen species (ROS)-driven oxidative damage in intervertebral discs could originate from cellular response to pro-inflammatory cytokines (Nasto et al., 2013). Reduced form of CoQ10 (ubiquinol) can effectively protect by reducing the initiating free radicals and preventing their propagation. The potent antioxidant property of ubiquinol presents a strong defensive mechanism against oxidative damage for all cells. The current study is comparable with the study of Hiebert, showing the beneficial effects of CoQ10 supplementation in treating various stress conditions, such as neurodegenerative diseases, congestive heart failure, ageing or cancer (Hiebert et al., 2012).

The mean disc wedge index of the animals was found to be significantly higher in the disc immobilized group B as compared to control group A. While the animals in group C and D showed insignificant increase in DWI and was close to control group A at the end of the experiment. This increased DWI in group B is in agreement with the study performed by Adams et al. (2000), which most probably is due to degeneration leading to reduced water content of the nucleus pulposus which causes NP to lose its ability to dissipate force. AF becomes responsible for absorbing a greater amount of force and becomes disorganized and vulnerable to tears and AF more affected posterior to the nucleus pulposus, resulting in higher reduction of disc height posteriorly. The disc degeneration leads to extreme outward bulging and complete radial fissures of the annulus, which allows posterior migration of nucleus pulposus. The insignificant increase in DWI in group C and D is because omega 3 and CoQ10 reduce inflammation and free radical injury respectively. This retards the process of degeneration in IVDs by down-regulating the inflammatory factors and scavenging reactive oxygen species leading to reduced degradation of ECM (Robert et al., 2007). Our study shows that omega 3 and CoQ 10 have significant potential in treating IVD degeneration and CoQ10 has better protective effect than omega 3 fatty acids.

CONCLUSION

In this study, radiological changes induced by immobilization in the intervertebral discs of the experimental rats and its reversal by omega 3 and CoQ10 were proven. Omega 3 and CoQ10 administration minimized immobilization induced degeneration of IVDs.

CONFLICT OF INTEREST

It is stated that there is no conflict of interest with

any individual, organization or any funding agency.

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