SUBCHRONIC TOXICITY OF PROTEOGLYCAN F IN RATS

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Proteoglycan not only reduces arthritis pain but also prevents complications, because it prevents joint damage, has anti-inflammatory effects. So, Proteoglycan is a solution to relieve pain and prevent osteoarthritis and osteoporosis effectively. The aim of this study is to investigate the subchronic toxicity of Proteoglycan F (proteoglycan from salmon cartilage) in experimental animals. The subchronic toxicity was carried out in Wistar rats according to the guidelines of the World Health Organization on medicinal herbs of origin. The rats were treated with the doses of 40 mg/kg/day and 120 mg/kg/day for a period of 90 consecutive days. After 90 days of treatment, Proteoglycan F did not cause significant dose-related changes in hematological parameters, renal and hepatic functions as well as histopathology tests. These results suggested that Proteoglycan F has no subchronic toxicity in experimental animals.

Keywords: Proteoglycans, salmon, subchronic toxicity, experimental animals, rats, osteoarthritis, OA

I. INTRODUCTION

Osteoarthritis (OA) of the knee is a disease that has the damage of all joint components such as cartilage, subcutaneous bone, ligaments, synovial membrane, in which the main lesion is in cartilage. The disease can occur in all ages, especially the elderly and appears in every country in the world, seriously affecting mobility, quality of life.[1] Knee joint is the largest joint of the body, the main force in movement. Improvement of movement is a very important goal in the treatment of osteoarthritis. It is necessary to slow down the degeneration process and ensure the movement of patients.

Proteoglycans are a major component of the extracellular matrix. They form large complexes, to other proteoglycans, to hyaluronic acid, and to fibrous matrix proteins, such as collagen. The combination of proteoglycans and collagen forms cartilage.[2] The loss of proteoglycans from articular cartilage is a hallmark of the osteoarthritic process. Changes, occurring in the pericellular region demonstrating a loss in metachromasy, suggest that depletion of specific proteoglycans surrounding the chondrocyte may be of significance in the early osteoarthritic lesion.[3] A few studies showed some evidence of the anti-osteoarthritis effects of proteoglycan. But no information on proteoglycan toxicity in experimental animals was reported.[4,5] This study was investigated to evaluate the subchronic toxicity of Proteoglycan F, which is extracted from salmon cartilage, in experimental animals.

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II. METHODS

1. Plant materials

Proteoglycan F (PF) appeared in powder, contained 26,1% proteoglycan extracted from

salmon cartilage. Sample was dissolved in water before oral administration.

A specimen of Proteoglycan F (Lot number 180829) was deposited at the ICHIMARU PHARCOS Limited Company, Japan.

2. Experimental animals

Normal healthy Wistar albino rats weighting between 160 g and 200 g were obtained from animal center of Dan Phuong, Ha Noi. The animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of the study. They maintained for 12 hours in light and dark cycle in a well ventilated house, with free access to food and water ad libitum.

All animals were treated according to the international regulation on the experimental animals treatments (ref.).

3. Experimental design

Subchronic toxicity study were carried out according to guidance of World Health Organization and Organisation for Economic Co-operation and Development.[6, 7]

A total of 30 rats were randomly divided into three groups housed separately in cages. The animals were daily treated by oral route with saline solution (control), and Proteoglycan F at doses of 40 and 120 mg/kg (representing the pharmacologically active dose, three times the pharmacologically active dose), for a period of 90 consecutive days.

Rats were weighed weekly and observed for behavioral changes, feeding and drinking habits and general morphological changes. At the end of the treatment period, blood samples were collected from all groups by saphenous vein puncture into EDTA sample tubes for hematological analysis (total red blood cells, hematocrit, hemoglobin concentration, total white blood cells and platelet count using automatic hematological analyzer Exigo - Boule Medical AB.) and into plastic sample tubes for serum generation for biochemical analysis (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol and creatinine using biochemical analyzer Erba Chem.). From each group, three rats were sacrified to harvest vital organs including the livers and kidneys. The organs were preserved in 10% formol-saline for histopathological assessment. Mortality in each treatment group was recorded during the 90 days treatment period of Proteoglycan F.

4. Statistical analysis

Results are expressed as Mean ± SD. Data analysis was carried out using Student's t test and paired t test using Microsoft Excel vers. 2013. Significance was considered at values of $p < 0.05$.

III. RESULTS

1. Effects of Proteoglycan F on Body Weight, Food and Water Intake

No significant differences in the average body weights were observed between the treated groups and the control group ($p > 0.05$) (Figure 1). Both control group and Proteoglycan F group with two doses did not produce a change on average weekly food and water intake in treatment (resulted in observation).

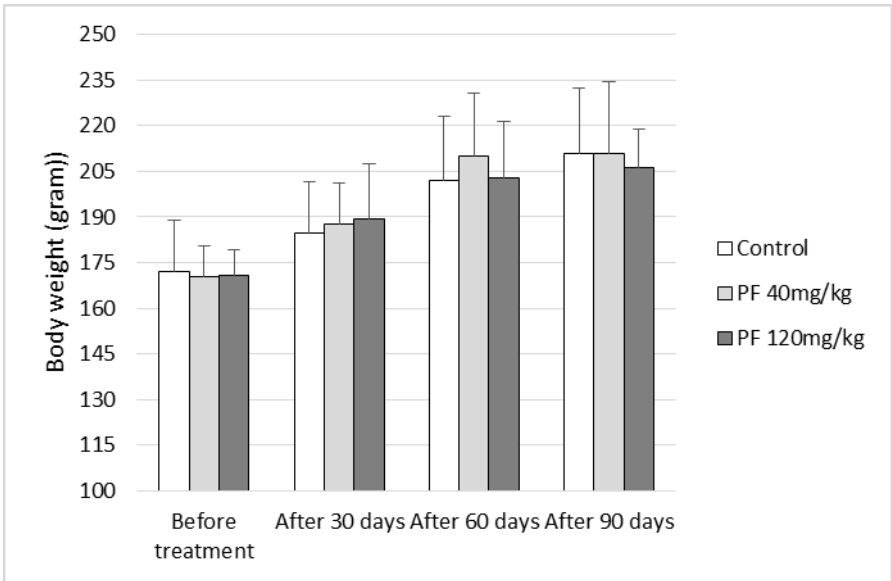

Figure 1. Effect on body weight

2. Mortality

In the subchronic toxicity study, there were no deaths in the control group and the Proteoglycan F (40 mg/kg and 120 mg/kg) treatment groups within treatment periods.

3. Effects of Proteoglycan F on Hematological Parameters

Proteoglycan F at two doses did not produce any significant effect on hematological parameters during period of administration (Table 1-3 and Figure 2-3).

Table 1. Effect of Proteoglycan F on total red blood cells

Days	Total red blood cells (T/l)		
	Control	PF 40mg/kg	PF 120mg/kg
Before treatment	7.77 ± 0.88	7.93 ± 0.56	7.91 ± 0.59
After 30 days	7.42 ± 0.45	7.35 ± 0.46	7.62 ± 0.26
After 60 days	7.19 ± 0.50	7.32 ± 0.62	7.56 ± 0.38
After 90 days	7.49 ± 0.79	7.33 ± 0.79	7.43 ± 0.52

Table 2. Effects of Proteoglycan F on total white blood cells

Days	Total white blood cells (G/l)		
	Control	PF 40mg/kg	PF 120mg/kg
Before	9.61 ± 2.65	8.67± 2.40	9.19 ± 2.79
After 30 days	10.55 ± 3.48	10.50 ± 2.52	9.40 ± 2.36
After 60 days	8.58 ± 2.13	11.13 ± 3.38	10.09 ± 2.81
After 90 days	9.60 ± 3.28	8.63 ± 1.92	10.35 ± 2.69

Table 3. Effects of Proteoglycan F on platelet count

Days	Platelet count (G/l)		
	Control	PF 40mg/kg	PF 120mg/kg
Before	352.70 ± 69.65	320.00 ± 97.44	318.10 ± 133.75
After 30 days	329.60 ± 41.64	286.80 ± 57.05	310.50 ± 37.15
After 60 days	366.60 ± 73.66	401.40 ± 132.71	299.10 ± 104.69
After 90 days	363.70 ± 77.62	412.30 ± 120.24	361.10 ± 82.43

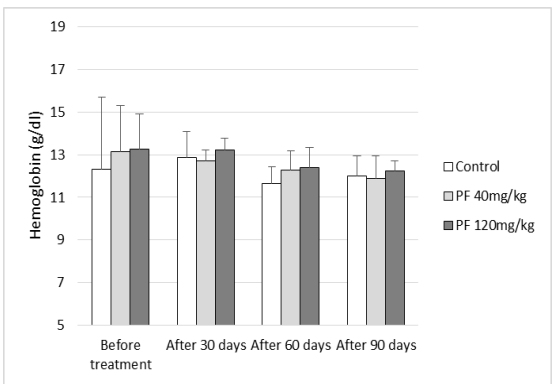

Figure 2. Effects of Proteoglycan F on hemoglobin

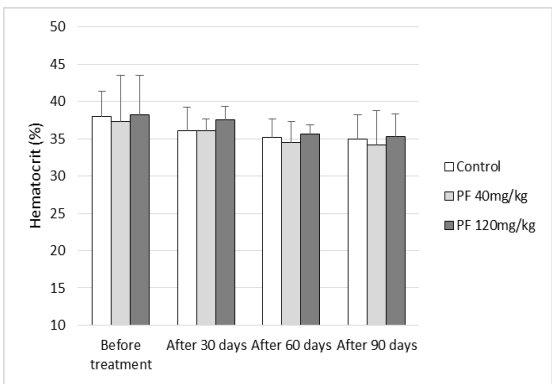

Figure 3. Effects of Proteoglycan F on hematocrit

4. Effects of Proteglycan F on Serum Biochemical Parameters

In this study, serum biochemical parameters were measured at 30, 60 and 90 days of treatment, but there were no changes over these parameters. (Table 4 has shown results at 90 days of treatment).

Table 4. Effects of Proteoglycan F on serum biochemical parameters at 90 days of treatment

Parameters	Control	PF 40mg/kg	PF 120mg/kg
Albumin (g/dl)	3.11 ± 0.44	3.30 ± 0.23	3.06 ± 0.51
Total cholesterol (mmol/l)	1.68 ± 0.30	1.89 ± 0.45	1.91 ± 0.28
Total bilirubin (mmol/l)	13.62 ± 0.69	13.18 ± 0.53	13.49 ± 0.66
Creatinine (mg/dl)	1.04 ± 0.11	1.02 ± 0.11	1.02 ± 0.09
SGOT (UI/l)	87.30 ± 21.28	86.50 ± 18.40	88.50 ± 11.69
SGPT (U/l)	58.70 ± 12.52	48.40 ± 12.79	50.30 ± 14.11

5. Histopathological examination

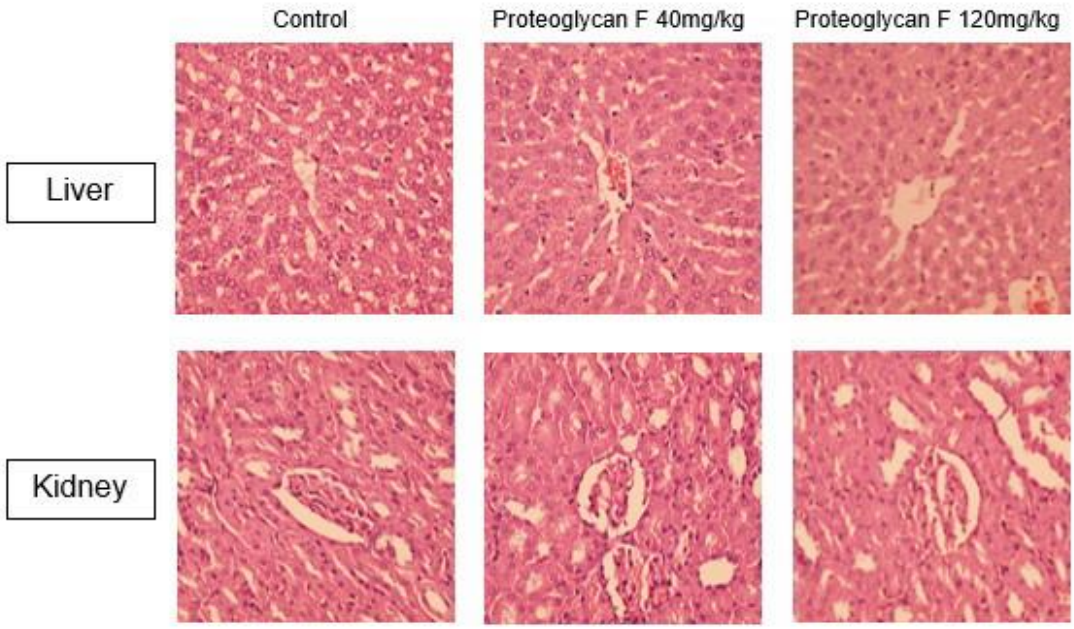

Figure 4. Effects of Proteoglycan F on histologic structure at 90 days. (Selected microphotographs HE x 400)

At the end of the treament period, no significant histopathological changes were observed during autopsy in all treatment groups. Figure 4 shows histological results at 90 days. There were no adverse histopathological effects observed in the liver of the control and Proteoglycan F treatment groups. The liver appeared normal with preserved hepatic structure, normal hepatocytes, and having eosinophilic cytoplasm and central nuclei. In the kidney , there were no adverse histopathological presentations observed in all the treatment groups and control group. No necrosis was observed. Normocellular glomerular tufts were displayed on a background containing tubules.

IV. DISCUSSION

OA is a chronic disease, so that the medications required a long-term use. The scientists are interested in the search for new drugs for OA management, and Proteoglycan is the one of the new choices. Proteoglycan is just a food supplement, therefore patients with OA have to take it in a long period to get the effects. Therefore, it is essential to evaluate the subchronic toxicity of this product.

The general condition and body weight change are the indicators that must be evaluated in subchronic toxicity studies.[7] During the study period, all rats in control and treatment groups had no changes in behavior, drinking and eating habits. The decrease of animal weight or the change in general condition during study may be the bad effects of testing samples. Weight gain was observed in all animals administered with Proteoglycan F. There was no statistically significant difference between the test groups and the control group, thus, showed that Proteoglycan F have less impact on the general condition and weight of rats.The body weight changes serve as a sensitive indication of the general health status of animals.[7]

The hematopoietic system is not only one of the most sensitive targets of toxic compounds but also an important index of physiological

and pathological status in animals. According to WHO, assessing more and more parameters of the hematopoietic system is more likely to accurately assess the toxicity of drugs.[6] Therefore, tests on some parameters such as the quantity of red blood cells, hematocrit, hemoglobin concentration, total white blood cells and platelet count of rats were used. After 90 days of treatment, the above indicators of rats in both treatment groups were not significantly changed compared to control group. It may suggest that Proteoglycan F did not cause toxic effects at these dose regimens in rats.

The liver plays a key role in many metabolic processes for not only itself but also for other tissues as well. When substances are taken into the body, they can be toxic to the liver, affect the liver functions. Therefore, when assessing the toxicity of drugs, studying the effect of drugs on liver functions is very necessary. To assess the extent of hepatocellular injury, the serum concentrations of liver-derived enzymes are usually quantified. Increasing levels of these enzymes are often associated with substances toxicity due to destruction of liver cells.[7] In our study, the concentrations of ALT (alanine amino transferase) and AST (aspartat amino transferase) of rats remained unchanged after 90 days of continuous Proteoglycan Fadministration. Besides, total bilirubin, albumin and total cholesterol are useful indices of the excretory functions of the liver. The results of the serum biochemical parameters of the animals treated with Proteoglycan F showed that no significant changes in ALT, AST, total bilirubin, albumin and total cholesterol at two doses. These indicate that Proteoglycan F had no deleterious effect on liver functions and damages. Furthermore, histopathological examination of the livers of the control group and all treated groups did not reveal any morphological differences.

The kidneys are the organ that helps the body pass waste as urine and filters blood before sending it back to the heart. The kidneys perform many functions, including: regulating and filtering minerals from blood and filtering waste materials from food, medications, and toxic substances, etc. Therefore, substances are taken into the body can cause toxicity to kidneys, affect the kidney functions.[8] Creatinine is the most stable protein component in the blood, almost regardless of diet or physiological changes, depending only on the ability of the kidney to be eliminated. When glomerular is damaged, blood creatinine concentration increases earlier than urea. Blood creatinine concentration is a more reliable and important indicator than blood urea, so it is currently used to assess and monitor kidney function.[9] In this study, creatinine concentration in peripheral rat blood of treatment with Proteoglycan F at two doses of 40 mg/kg/day and 120 mg/kg/day had no change compared to the control group after 90 days. In addition, the results of kedney histopathological examination of Proteoglycan F treatment rats showed normal structure and absence of any gross pathological lesions. The results of the study showed that Proteoglycan F did not affect the kidney functions.

Overall, the findings of this study indicate that no significant differences were observed concerning blood profile, biochemistry parameters. Unfortunately, there are no studies have been performed about toxicity of Proteoglycan, so that we not able to compareour results. These observations indicate that Proteoglycan F at the dose of 40 mg/kg/day and 120 mg/kg/day can be used for further pharmacological activities.

V. CONCLUSION

This study demonstrated that Proteoglycan F (extracted from salmon cartilage) at the doses of 40 mg/kg/day and 120 mg/kg/day did not cause any toxic signs or evident symptoms at subchronic toxicity by oral route in rats.

Acknowledgments

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Conflicts of interest

The authors declare no conflict of interest.

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