

The Effects of Glucosamine Sulphate on Mice Skin

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ABSTRACT

Glucosamine is an amino monosaccharide derived from cellular glucose metabolism and it is a simple component or "building block" of more complex molecules. Glucosamine was considered to be an effective treatment for many joint diseases especially osteoarthritis.

It is believed that glucosamine maintain healthy joint functions and rebuild damaged joint cartilage, tendons, ligaments and other connective tissue. It does this by stimulating the production of glycosaminoglycans (GAG's) which are the structural components of cartilage and connective tissue else where in the body.

Twenty male and twenty females adult mice (weighting between 23.3gm to 27.2gm), were divided into two equal subgroups (control and experimental); the drug was given at noon three hours after starvation it had been grinded and mixed with food and given as a single oral dose of 350mg/kg body weight per day for 35 successive days. Histological examination and statistical analysis of multiple sections of ventral and dorsal skin of male and female mice and of both subgroups were done by using hemotoxyline and eosin stain.

The results reveal that there was increment in the number of fibroblast which was more obviously seen in the ventral skin of the treated animals. This study confirms practically that glucosamine sulphate induces significant structural changes in the skin of mice.

Using glucosamine sulphate clinically for medical conditions rather than arthritic diseases is now the target of most recent researches, its ability to decrease wrinkles in the aged skin and promotion of wound healing with less scar tissue is consider to be a light for dermatologist and plastic doctors, plus its effect to rebuild any aged, injured and diseased tissue in the body.

Key words: Glucosamine, glycosaminoglycans, fibroblast

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INTRODUCTION

Glucosamine is a substance found naturally in the body, it is an amino sugar that is thought to play a role in the formation and repair of cartilage, and it is a nutritional supplement that is derived primarily from shrimp shells. It appears to help maintain healthy joint function as well as help rebuild damaged joint cartilage, tendons, ligaments and other connective tissue. It does this by stimulating the production of glycosaminoglycans (GAG's) which are the structural components of cartilage and connective tissue. In addition, glucosamine sulphate is thought to contribute sulfur, an essential nutrient for joint and connective tissue. ^(1 and 2) Glucosamine sulphate has several actions. It serves as a precursor for, and inhibits the degradation of proteoglycans (the ground substance of articular cartilage); it rebuilds experimentally induced cartilaginous damage; and it has chondroprotective and antiarthritic effects. ^(3 and 4) The clinical use of glucosamine supplementation may extend beyond the treatment of osteoarthritis. It may also required for the synthesis of other glycosaminoglycans that are integral components of the basement membrane below the skin and intestinal tract lining and blood vessels and as reviewed by McCarty, glucosamine supplementation can be used to enhance wound healing (e.g., post-surgical),

through its effects on stimulating the synthesis of hyaluronic acid. ⁽⁵⁾ A study, published recently found that glucosamine plus amino acids from collagen and antioxidants reduce wrinkles by 34 per cent in five weeks compared to placebo. Alterations in the skin's vitality and appearance, i.e. anti-aging, are positively influenced by collagen, antioxidants and glucosamine supplementation. ⁽⁶⁾ From previous review it has shown that medically, glucosamine sulphate in the present time is widely used for many purposes especially for the treatment of osteoarthritis and joint diseases. Also it has been recorded that a lot of biological, biochemical and pharmacological studies has been done to evaluate the effect of this drug on different organs and tissues in man and animal.

However, no attempt has been made to find out the structural effect of this drug on the skin, and the usage of glucosamine as anti-aging compound, therefore we planned to investigate this histological effect of glucosamine sulphate on mice's skin from different sites (ventral and dorsal), and by using a single daily dose of 350mg/kg body weight.

MATERIALS & METHODS

The drug used in the experiment was glucosamine sulphate tablets, these tablets

were kept in a cool dry place and protected from light. Treated mice were given the drug mixed with their food in one daily dose. The tablets weighted using the Mettler H54 A.R. microbalance (Karl Kolb-West Germany) to estimate the recommended dose which was 350 milligram / kilogram of mice body weight, and grinded by manual grinder.

Meal was prepared by manual grinding of the commercial pellet and mixed with the grinded drug; this was given at noon 12.00 am three hours after starvation. Meal was given daily for successive 35 days.

Forty adult albino *Mus. musculus* mice (twenty males and twenty females) weighting between 23.3gm to 27.2gm. After the animals had been anaesthetized with chloroform and sacrificed.

Dissection was done as fast as possible while the heart was still beating; skin was taken from both ventral (abdominal) and dorsal (back) regions.

Using dissecting set, the skin was separated from the underlying tissue, trimmed to the size of (2×2cm), and hair was cut using special scissor, taking care not to injure the tissue. This specimen was taken and used for paraffin sections.

Readings were obtained from tow regions (ventral abdominal and dorsal skins). From each region five random serial sections were obtained, each section was of five micron (5

µm) in thickness. Ten random readings from each section were obtained i.e. fifty readings for each region, thus one hundred readings for each animal were studied. The numbers of fibroblasts were counted from the dermal-epidermal junction to hypodermis per field of microscopic view, this was done using light microscope, under a magnification power of (X800), and the mean of them was obtained.

Results were presented in simple measures of mean $\pm$ S.D. and 95 per cent confidence interval for significance. The significance of differences among the quantitative variable was assessed using one-way analysis of variance (ANOVA). A (P) value of less than (0.05) was considered significant.

RESULTS

The dermis of the treated animals showed high cellular content. There was a remarkable high number of mature fibroblast in both ventral and dorsal skins of treated animals (Figures 1, 2&3).

The control shows less collagen fibers with less cellular contents especially fibroblast (Figure 4).

The active fibroblast was long with oval abundant nucleus which contains one to two nucleoli; the fibroblast were condensed and elongated parallel to the direction of the collagen fibers (Figures 1, 2&3).

The cytoplasm was greatly increased in volume with deep basophilic color; the fibroblast had fine cytoplasm processes extending into the matrix to meet up with those of other fibroblasts (Figure 4).

The ventral skin of male mice showed a significant differences in the number of fibroblast in experimental group [mean difference = 20.761, 95% CI = 20.274 to 21.248], comparing it with control group which was [mean = 6.392, 95% CI = 6.234 to 6.55] (Table 1) (Figure 5).

Also sections of dorsal skin of the experimental group showed a significant increment in fibroblast count [mean = 17.16, 95% CI = 16.7 to 17.618] comparing it with the control group which was [mean = 6.047, 95% confidence interval equal to 5.871 to 6.223] (Table 2) (Figure 6).

The ventral skin of female mice shows a significant differences in the number of fibroblast in experimental group [mean = 19.0, 95% confidence interval = 18.501 to 19.499], it was higher than that of control group [mean = 5.872, 95% confidence interval = 6.234 to 6.55] (Table1) (Figure 5).

Similar to that of ventral skin the number of fibroblasts in dorsal skin of experimental group was significantly increased [mean = 21.64, 95% CI = 21.14 to 22.14], when it was

compared with control group [mean = 6.13, 95% CI = 5.94 to 6.31] (Table 2) (Figure 6).

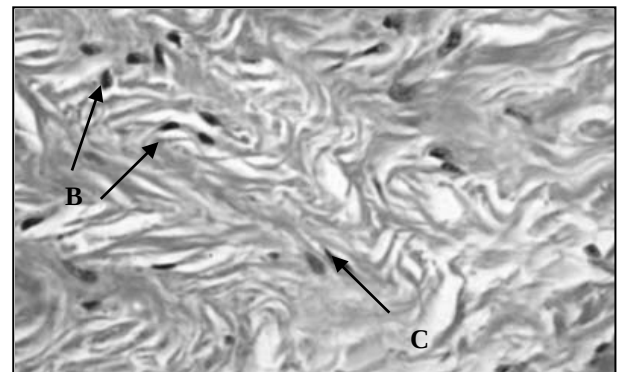


Figure (1): Section of adult female mice (ventral skin), treated with GS, demonstrate thick collagen fiber with high cellularity, large number of fibroblasts (B) and scattered fibrocytes (C). H&E stain, X400.

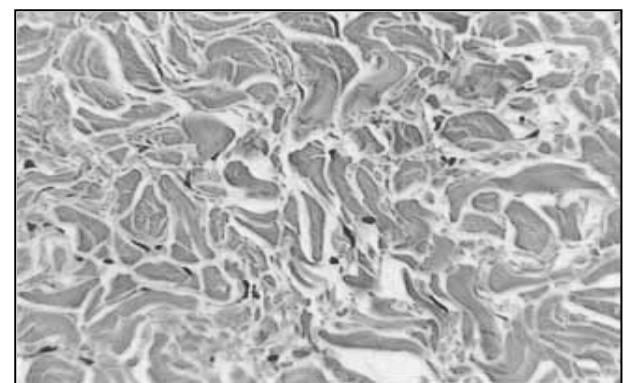


Figure (2): Section of adult male mice (dorsal skin), treated GS, demonstrate thick collagen fiber with high cellularity, large number of fibroblasts and scattered fibrocytes. H&E stain, X400.

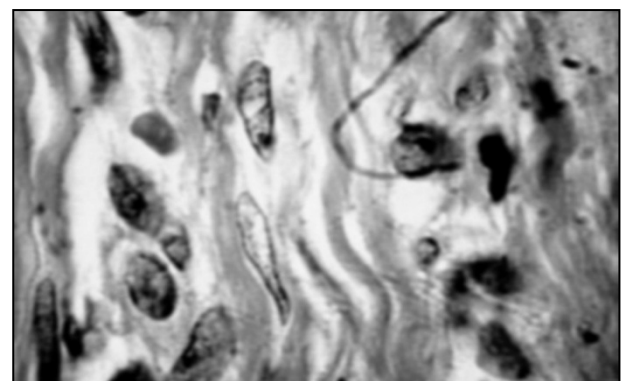


Figure (3): Section of adult female mice (ventral skin), treated GS, demonstrate the fibroblasts are condensed, elongated, and the

cytoplasm is basophilic in color. Fibrocytes are fewer and small in size. H&E stain, X1000.

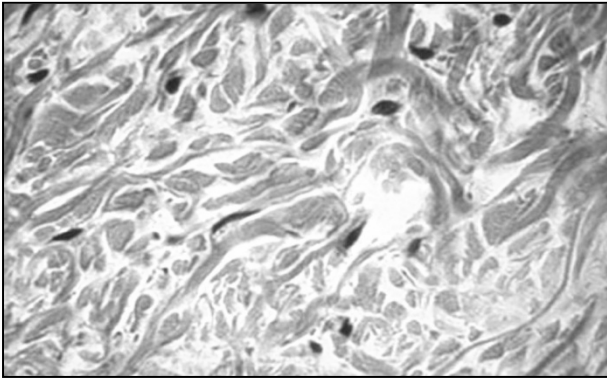


Figure (4): Section of adult male mice (dorsal skin), control, demonstrate thin collagen fibers with less cellularity, small number of fibroblasts H&E stain, X400.

Table (1): Number of fibroblast in control and treated male mice with Glucosamine Sulphate.

	Number of Fibroblast (Mean $\pm$ SD)		P value
	Control (n=100)	Experimental (n=100)	
Ventral Skin	6.392 $\pm$ 2.552	20.761 $\pm$ 7.857	p< 0.00001
Dorsal Skin	6.047 $\pm$ 2.839	17.16 $\pm$ 7.397	p< 0.00001

Table (2): Number of fibroblast in control and treated female mice with Glucosamine Sulphate.

	Number of Fibroblast (Mean $\pm$ SD)		P value
	Control (n=100)	Experimental (n=100)	
Ventral Skin	5.872 $\pm$ 2.658	19.00 $\pm$ 8.051	p< 0.00001
Dorsal Skin	6.13 $\pm$ 2.992	21.64 $\pm$ 8.079	p< 0.00001

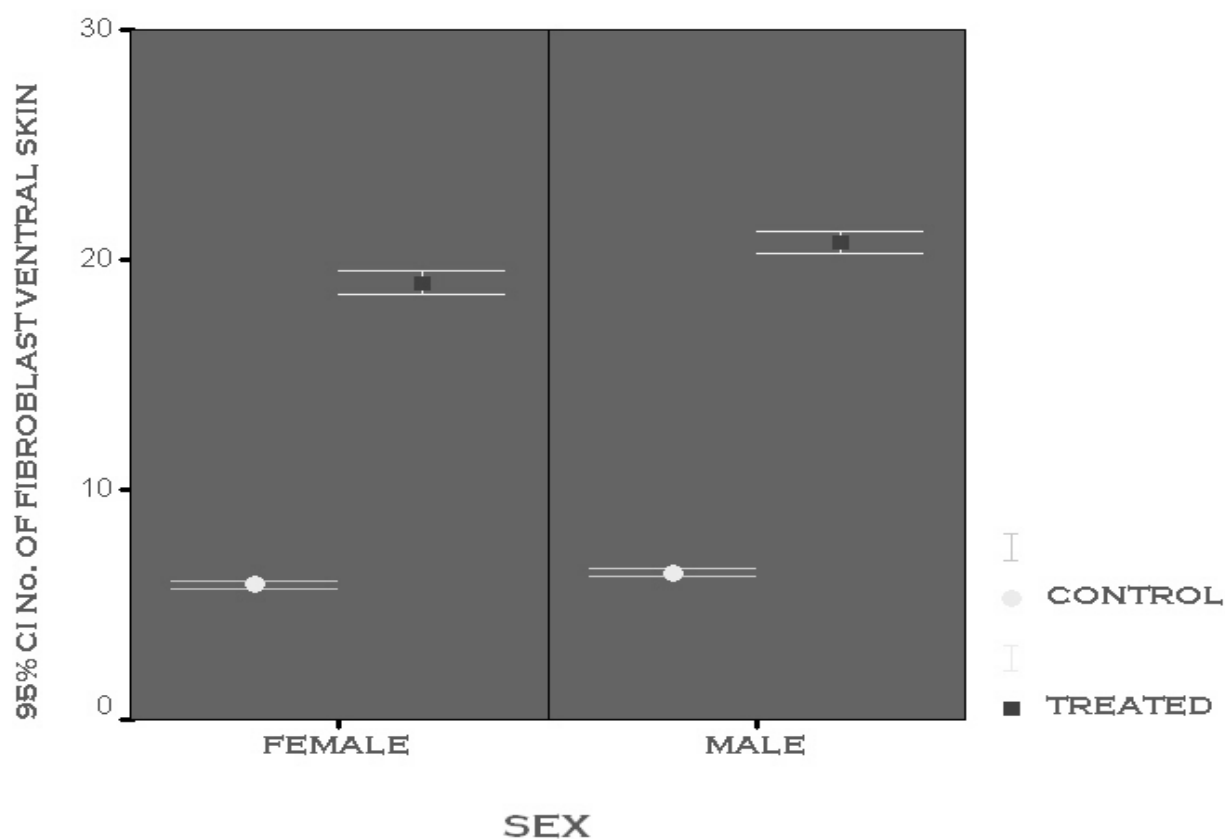
**Figure (5):** Diagram represents the 95% confidence interval of fibroblast count in ventral skin of male and female adult mice.



Figure (6): Diagram represents the 95% confidence interval of fibroblast count in dorsal skin of male and female adult mice.

DISCUSSION

During aging, there is gradual and progressive slowing of cellular turnover and regeneration, these results in thinning of the skin which is due to the flattening of the ridge like dermal-epidermal interface. ⁽⁷⁾ Our treated animals had increased the activity of dermis by increasing the number of active fibroblast, which might support the anti-aging effect of glucosamine sulphate on the skin.

The skin is another tissue which can be a target organ for glucosamine sulphate administration, since it can stimulate the fibroblast to produce more collagen fibers. ⁽⁶⁾ In support to our hypothesis is the review made by McCarty, which showed that glucosamine sulphate can be used to enhance wound healing (e.g., post surgical), through its effect on stimulating the synthesis of hyaluronic acid. ⁽⁵⁾

In particular, senescent dermal fibroblasts over-express metalloproteinase activities

that may explain the age-related atrophy of extra-cellular matrix architecture. ⁽⁸⁾

Skin extracts and newly synthesized collagen from fibroblast cultures derived from both old and young donor groups showed the same ratio of collagen III to collagen I. The data suggest that fibroblasts maintain a uniform level of collagen production, composition and modification independent of the age of the donor. ⁽⁹⁾

Application of glucosamine sulphate enhances collagen formation in the dermal layer which is produced by the active fibroblast; therefore the glucosamine sulphate might have an effect on the rapid collagen turnover, and thus has led to collagen accumulation in the dermis. ⁽⁹⁾

The effect of glucosamine sulphate showed a significant increment and similarity between male and female regarding ventral and dorsal skins concerning the number of fibroblasts in both sexes, the increased number of fibroblast is due to the condition of the active state of the skin and the stimulation of more fibroblast activity.

Since active fibroblast were more numerous in experimental animals, that lead to high production of collagen and elastic fibers and more ground substance

(mucopolysaccharide) and this is might be due to the direct effect of the administered glucosamine. Fibroblasts exhibit a

biosynthetically activated persistency, which persists for several years,⁽¹⁰⁾ which might explain the increased dermal thickness, the amount of collagen, elastic fibers, and ground substances.

CONCLUSION

As a final consideration, the decline in glucosamine sulfate synthesis with age may imply that a prudent anti-aging strategy is to use a low to moderate dose of glucosamine sulphate as an anti-aging strategy beginning at 45-50 years of age, this intervention may help to prevent or minimize the development of osteoarthritis changes to our joints, helping to preserve quality of life to a significant degree and will cause alteration in collagen, elastin, and glycosaminoglycan contribute to cutaneous changes seen in aging skin. Stimulation and hyperactivity of the fibroblast will lead to increment in the dermis thickness and the renewing of the skin tissue with increasing vascularity and high cellular contents. Using glucosamine sulphate clinically could be pointed to decrease the wrinkles in the aged skin.

In addition to increase the ability of the skin to repair itself with faster wound healing, so the skin will become more resistant to injuries and damage.

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