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A Chronic Toxicity Study of Oral Administration of Collagen-α® Supplement using Pregnant Rabbits

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Abstract

Since collagen derived products are highly used in food supplements due to its bioactive properties by influencing cellular and tissue health, flexibility with high effect of repairing and adaptation. It is necessary to carry out safety requirement of an oral chronic toxicity assessment. This study investigated the adverse effect of long term oral administration of collagen-® in pregnant rabbits. We have conducted a thirty days randomized controlled trial in pregnant rabbits. Rabbits were randomized into two groups; Group 1 (control group) were administrated with 1 ml of normal saline PO, SID while Group 2 (collagen-α group) were administered with 1 ml of collagen-α® PO, SID for one month. Bodyweight gain, viscera weight and histopathological evaluation of the individual rabbit were recorded. The results revealed that the body weight gain and visceral tissues weight in the collagen-α group were significantly lower than the control group. Moreover, various histopathological changes were recorded in heart, lungs, liver and kidneys of the collagen-α group when compared with the non-significant changes that noticed in the control group. Under the conditions of the current study, a high risk of chronic toxic effect was observed in pregnant rabbits inoculated with 1 ml of oral dose of collagen alpha supplement. This result suggests that the adverse effect of collagen derived supplements is overbalancing the benefits.

Key words: *Collagen-α, Rabbits, Food Supplements, Weight Gain,*

Introduction

Collagen is considered as the most plenteous structural protein of living organism including vertebrates and invertebrates that constituting approximately 30% of total animal's proteins [1]. It's a complex alloy of the extracellular matrix of variable members of diverse families of protein exhibiting various physiological functions and structural integrity. Fundamental cell biology such as proliferation, migration cytoskeletal organization, apoptosis and differentiation are essentially regulated by collagens [2]. Moreover, collagen is the major structural element of all the connective tissues, and presents in the interstitial tissue of parenchymal organs. Thus as a results of its fibrillar structure, collagen is contributing the extracellular scaffolding which subsequently safeground the stability of tissues and organs and maintain their structural integrity [3,4].

Around 28 types of collagen have been yet identified, which is composed of 46 distinct polypeptide chains [5]. The collagen has triple helix characteristic polypeptide chains. It probably formed by three identical homotrimers chains represented as collagens II, III, VII, VIII, X and others; or heterotrimers as in types of collagen I, IV, V, VI, IX, and XI [6,7]. Permanent processes of collagens exchange take place in the body during human life; replaced of the old fibrils by new one. In the young, production of the collagen and its degradation takes place in dynamic balance, while during tissues maturation, degradation is occurring more intensive [8,9,10]. Smoking cigarettes, stress, UV radiation and unhealthy diet lead to the degradation of natural structure of collagen and to earlier senility [11].

The sources of collagen mostly came from wild animals (mainly porcine and bovine) and birds [5]. Due to their functional and nutritional properties, the collagens and "collagen-derived" products are recently widely used as food supplements, in pharmaceutical, and cosmetic industries [1]. However, collagen supplements have clinically given their beneficial functions, especially maintaining and improving the connective tissues fore instance of; joints, skin dermis, ligaments, tendons, and

blood vessels [3,5,12].

The exploration of the functions of the different collagen types is contributes to a better understanding the progress of the diseases as well as the embryonic and fetal development processes [3]. Little is knowledge's about the role of collage defect as a source of abanants diseases/conditions among both human and animals in Iraq that threating its utilization in daily life food-supplement as well as the chronic toxicity effects of these products. Therefore, the current study was designed to evaluate the effects of collagen-α as a food supplement on clinical and cellular changes using pregnant rabbits as a useful group study model.

Material & Method

Animals and Experimental Design

This study was conducted as a thirty-day prospective blinded assessment trail on female laboratory rabbits during November 2018. Twelve pregnant rabbits weighting between 960-1100 grams were housed in controlled environmental conditions at Animal House, College of Veterinary Medicine, Basrah University, Iraq (temperature of 25-28 □C). The rabbits were divided randomly into two equal groups; Group 1 and 2. The group 1 (control group) were orally administrated with 1 ml of normal saline while group 2 (collagen-α group) were inoculated orally by 1 ml of collagen-α® (CH-Alpha®, GELITA Thailand) SID for 30 days. Our experiment was approved following the roles and the guidelines of the Scientific and Researches Committee of the College of Veterinary Medicine, University of Basra, Iraq.

The body weight (BW) of each rabbit was recorded separately at the onset and at the end of the experiment. Weight gain was calculated following the formula (BW gain=Final BW-initial BW).

At the end of the study, all rabbits of both groups were euthanized. Organs such as the heart, liver and kidneys were immediately severed and weighted and fixed in 10% formalin buffer at for 24 hrs. The Specimens were dehydrated through a graded series of ethanol and xylene prior to paraffin embedding and staining with hematoxylin and eosin (Harris H&E) finally mount with DPX [13].

Statistical analysis was performed using SPSS version 22.0 for Windows. Independent "T" test analysis of variance with Tukey test was used to evaluate the differences among the two groups with regard to the study variables. Measuring of body and visceral weight are expressed as mean ± standard deviation (SD). A value of $P < 0.05$ was considered statistically significant.

Results

The weight of the pregnant rabbits of the treated group (collagen-α group (was significantly lower than in control group. However, a significant ($P < 0.05$) decreases in the weight of the kidneys, heart, lungs and liver were recorded in the treated group as comparasion with the control group (Table 1).

The most predominant changes of the renal tissues of the treated group are closure renal tubules that could be due to degeneration hemorrhage, and the vacuolation of the renal tubules when compared with the control group (Figures 1, A and B). Moreover, the vacuolation of the cardiocyte, hyperatrophy and spaces hemocedrosis were observed at the heart treated group (Figures 2,A and B) in contrast of control group. Thickening of alveolar wall, dilated of bronchiole and serous exudates at the lumen are most common cellular changes in the lungs tissues of the treated group comparing to normal lungs of control group (Figures 3, A and B). Finally, investigation of the tissue sections of the liver of the treated group showed moderate to severe congestion in central vein and fibrosis and swelling of hepatocyte) in comparation with control group (Figures 4,A and B).

Table (1): The effect of collagen-α on body weight, gain and relative weights of pregnant rabbits internal organs

Criteria Groups	Initial BW (g)	Final BWt(g)	Weight gain (g)	Liver Weight (g)	Kidneys Weight (g)	Heart Weight (g)	Lungs Weight (g)
G 1 Control (n=6)	1045.00 ±96.78	1365.00* ±62.34	250.00* ±28.28	67.75* ±1.89	9.24* ±0.24	54.50* ±3.15	84.50* ±5.06
G2 (collagen-α) (n=6)	990.00 ±30.43	1127.75 ±48.44	157.75 ±36.88	60.75 ±2.21	8.35 ±0.31	45.50 ±3.10	74.00 ± 6.05

***Significant differences (p<0.05). BW: Body weight**

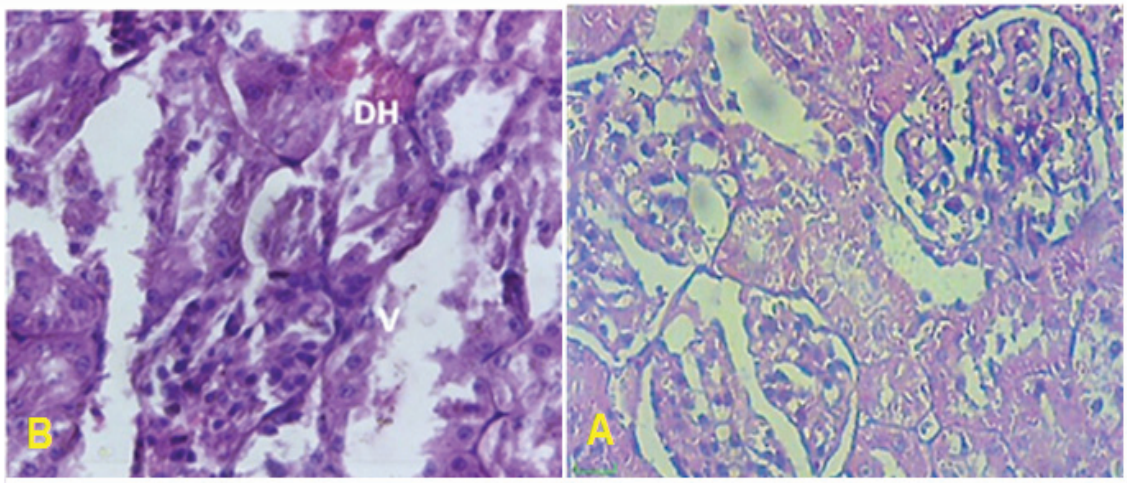

Figure 1: Cross section of kidney: A, in control group showing normal tissue, B. Treated group showing degeneration hemorrhage (DH) and Vacuolation (V).
H&E 400X

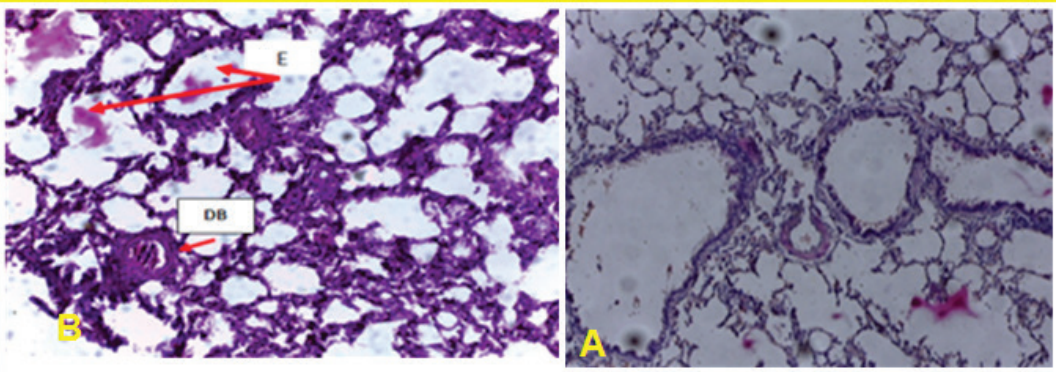

Figure 3: Across section of lung A. control group showing normal bronchiole and normal alveolar sac, B. Treated group showing dilated of bronchiole (DB) and exudates (E) into the lumen
H&E 100 X

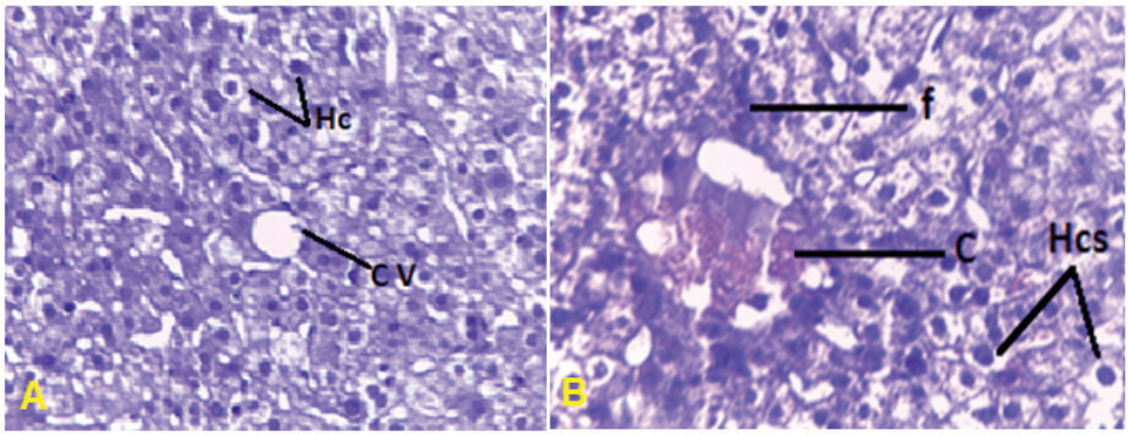

Figure 4: A cross section of liver, A. control group showing normal hepatocyte and central vein (CV) B. treatment group showing swelling of hepatocyte (Hcs), fibrosis (f) and congested (c) of central vein (CV) H&E 400X

Discussion

With the numerous bioactive properties, collagen derived products have long been used in food supplements and also in pharmaceutical products for maintaining the connective tissues. One of its clinical applications is for treatment the disorders of cartilages and skin [14]. Moreover, collagen and gelatin supplements could influence cellular and tissue health, flexibility with high effect of repairing and adaptation [15]. Commercial collagen products have been generally recognized as "saved" and approved for human consumption by the US FDA (16). Despite the collagen supplements appear to be with low risk, little data are available; recovery from injury, functional benefits, and the negative effect in athletes are also not known [15]. However, little literature exists that demonstrates the adverse effect of collagen supplements on visceral organs. In the current study, we have reported that the long term oral supplementation of collagen (collagen-α®) reduces weight gain companied with occurrence of cytopathic changes in heart, liver, lungs and kidneys of pregnant rabbits. To the best of our knowledge, this is the first chronic-toxicity study of collagen supplements using pregnant rabbit in Iraq.

The results of our study have suggest that the using of collagen-α® as a supplement consequently led to decrease weight gain as a results of its "Anti-Obesity" effect through regulation the metabolism of lipids [17]. Additionally, post mortem examinations of liver, lungs, kidneys and heart were also correlated to the results of the body weight gain findings. In contrast, Schauss et al., have conducted an acute and subchronic toxicity studies in rats that orally administrated by type II collagen derived from hydrolyzed chicken sternal cartilage. Regarding to acute and subchronic toxicity, all the rats were exhibit normal body weight with no significant histopathological changes throughout the study [18]. Collagen with hyaluronic acid and elastin have key role in providing elasticity and integrity to the organ. Collagen represents a family of 28 different proteins [19] which account for 30% of the total protein mass in the human body and play a pivotal role in the structure of several tissues, such as skin and bones, providing rigidity and integrity [3].

Despite the fact that there are no previous reports available on the hepato-toxicity induced by oral inoculation of food-derived collagen [20], our records have demonstrated histopathological alterations in liver after 30 days of collagen feeding. Similarly, Woo et al., have been mentioned that the collagen-fed mice had a lower level of hepatic Triglyceride; which was consistent with liver histological results. The Triglyceride lowering effect of collagen suppressed adipose tissue differentiation leading to diminish the adipocytes of collagen-fed mice [17]. These findings were in accordance with those of a previous related study, in which the intake of collagen peptide has decreased fatty acid synthesis and increased β-oxidation in the liver of mice [21].

The most dominant histopathological changes in kidneys were found in closure of renal tubules of collagen-α®-group; degeneration hemorrhage and vacuolation were observed. This cyto-nephrotoxicity is closely associated with the chronic effect of high protein renal filtration [22, 23]. Nevertheless, rats cold exhibit a renal hypertrophy at a dose rate of hundred times of the recommended daily collagen intake [24]. Regarding to the renal toxicity impact of collagen peptide, renal hypertrophy could be induced in growing rats following administration of porcine skin derived collagen peptide [25].

In the present study, we report that the oral supplementation with specific alpha collagen has adverse effects if be taken unnecessarily, it caused the vacuolation of the cardiocyte, hypertrophy and spaces hemocedrosis in heart as a result of high concentration of collagen (high protein diet). However, cardiac arrhythmias [26], myocarditis and cardiac atrophy [27] have been observed in individuals who after prolong receiving a low calorie and high protein (collagen) diet as a treatment of obesity.

In the present study, histopathological evaluation of lungs in collagen-α®-group showing thickening of alveolar wall, dilated of bronchiole and serous exudates into the lumen that could be due to accumulation of fluids as a result of circulatory impairs or as a sequel of heart damage. Moreover, inflammatory cell foci and emphysema were the most dominant lesions in lunges of rate after a 24-month feeding with marine collagen peptides [20]. Thus, no marked deleterious effects of collagen from bovine skin have seen in various animal studies except for local irritation when parenteral administrations are applied [28].

CONCLUSION

We provide a clinical trial of the oral chronic toxicity of specific collagen (collagen-α®) on pregnant rabbits for the first time in Iraq. Our data demonstrates that the prolonged oral administration of the specific collagen supplements can induce harmful cellular effects in the target organs and reduce weight gain. Despite the study's size and limitation, the results suggest that the high risk of adverse effect of collagen derived supplements is outweighing the benefits. Further investigations are needed to clarify the role of collagen-α® in other organs such as muscles, bones and cartilages as well as evaluate the related biochemical parameters. It will be interesting to compare different collagen sources regarding to their metabolic and adaptation effects.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine, University of Basrah and all experiments were carried out in accordance with approved guidelines.

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