Determining the effectiveness of Collagen and its therapeutic role in the immunological standards for laboratory animals induced by Pseudomonas aeruginosa

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ABSTRACT

The study was conducted in the laboratories of the Department of Food Science, College of Agriculture, Animal House, College of Veterinary Medicine, University of Tikrit for the period from 15 April to 22 May 2018, in order to determining the effectiveness of Collagen and its therapeutic role in the immunological standards for laboratory animals induced by Pseudomonas aeruginosa, as well as measuring the weights of some internal organs (heart, brain, liver, kidneys, spleen, and thyroid). The results showed that oral administration of standard and laboratory chicken collagen did not cause a significant change in the relative weights of the internal organs (heart, liver, kidneys, and thyroid gland), but it caused a significant decrease in the total number of granulocytes. While there was a significant increase in the percentages and numbers of lymphocytes, it was also found that oral administration of collagen caused a decrease in the levels of immunoglobulins IgG, IgM and IgA.

Keywords: collagen, P. aeruginosa, white blood cells, IgG, IgM.
Research paper from PhD thesis for the first author

تحديد فاعلية الكولاجين ودوره العلاجي في المعايير المناعية للحيوانات المختبرية مستحثه الإصابة ببكتريا *Pseudomonas aeruginosa*

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الخلاصة

أجريت الدراسة في مختبرات قسم علوم الأغذية / كلية الزراعة، والبيت الحيواني / كلية الطب البيطري -جامعة تكريت للمدة 15/ نيسان لغاية 22/ ايار/ 2018، لتحديد الدور العلاجي للكولاجين في بعض المعايير المناعية في اناث الجرذان المستحث الإصابة ببكتريا *Pseudomonas aeruginosa*، فضلاً عن تقدير اوزان بعض الأعضاء الداخلية (القلب، الدماغ، الكبد، الكلى، الطحال والغدة الدرقية). بينت النتائج ان الإعطاء الفموي من كولاجين الدجاج القياسي والمختبري لم يسبب تغير معنوي في الأوزان النسبية للأعضاء الداخلية لكل من القلب والكبد والكلى والغدة الدرقية، الا انه سبب انخفضاً معنوياً في العدد الكلي لخلايا الدم البيض وخلايا الدم البيض الحبيبية Granulocytes، في حين كان هناك ارتفاع معنوي في نسب واعداد الخلايا اللمفاوية Lymphocytes، كما تبين ان الاعطاء الفموي من الكولاجين سبب انخفاض مستويات الكلوبيولينات المناعية IgG، IgM وIgA.

الكلمات المفتاحية: كولاجين، *P. aeruginosa*، كريات الدم البيض، IgG، IgM.
البحث مستل من أطروحة دكتوراه للباحث الأول.

1. INTRODUCTION

Collagen is considered the main protein involved in the formation of tissues, bones, cartilage, and tendons. It is also the main component of skin proteins, classified as one of the most abundant types of fibrous proteins in the human body, where it constitutes approximately 30% from the total protein content of the body protein, and it forms 80-90% from the components of the skin. Collagen proteins included nineteen amino acids in their composition, the most important of which are Glycine (Gly), Alanine (Ala), Proline (Pro) as well as lower percentages of Hydroxyproline (Hyp) (8). Collagen varies according to confiscation, where there are more than 27 different types of it but the most important of them are the three types, the first, second and third (Type I, II

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and III) which are the most abundant and important for the human body (10). Pseudomonas aeruginosa belongs to the Bacteria Kingdom and the division of Gracilicutes. Scotobacteria described by a Pseudomonadales order and Pseudomonadeceae family according to the scientist classification (13). It is a Bacillus and aerobic Gram-negative bacteria generally spread in water and soil, It has the ability to produce colored materials (pyocyanin) with glowy and bluish-green color or yellowish-green color, the ideal temperature for its growth is 37 °C (7). It was found that these bacteria are the main cause for suppression of the burns that claimed the lives of many burn patients as a result of Septicemia, where they are found on the surface of the skin, which makes them easy to penetrate through the skin, causing many infections to wounds and burns (16). Because of the few studies that clarify the roles of bio-collagen, the study aims to determine the effectiveness of Collagen and its therapeutic role in the immunological standards for laboratory animals induced by Pseudomonas aeruginosa.

2. MATERIALS AND METHODS

Laboratory animals

A Thirty of an adult healthy female of Western Albino Rats were used to complete the experiment after examining them and making sure that there was no disease in it by the specialized veterinarian. The ages of the animals ranged between (50-45) days and weights of (120-168 g) obtained from the animal house belonging to the College of Veterinary Medicine, Tikrit University. Experiment animals were placed in metal cages of appropriate dimensions to the extent that would ensure their freedom of movement with the use of sawdust to cover the floor of the cage, in order to secure the maximum amount of moisture absorption. The sawdust was changed periodically every day to keep the cage clean. It was also emphasized that drinking water should be kept inside sterilizable plastic bottles. For the duration of the experiment, the animals were subjected to standard laboratory conditions in terms of ventilation, 20-25 °C, 14 hours lighting and 10 hours dark. All ethics of scientific research on experimental animals have been complied with according to the Experiment Animal Care Manual.

Preparing bacterial isolates

Pseudomonas aeruginosa isolate was obtained from a bacteriological laboratory at the Medya Diagnostic Center in Erbil.

Confirming the diagnosis of isolates:

The type of bacterial isolates was confirmed by developing Pseudomonas aeruginosa isolate on culture media (Cetrimide agar) and incubating at 35 ° C for 24 hours and observing changes in the color of the culture media.

Preparation of Bacterial Inoculum

The bacterial inoculum was prepared by collecting cells from the growing implant for 24 hours and adding it to Tryptone Soy Broth in volumetric flasks with the capacity of (250 ml), Bacterial numbers were calculated by calibrating with the tube at a concentration of 0.5 from the standard MacFarland solution to obtain the bacterial numbers for dosing at 1.5 x 810, The animals were given 1 ml of the oral bacterial inoculum using a dosing machine to ensure the inoculum was delivered to the animal's stomach.

Preparation of Collagen suspension

standard chicken collagen and laboratory chicken collagen used through dissolving 7 mg of collagen per 1 ml of distilled water to obtain the suspension that was dosed with an amount of (1 ml/animal).

Design of the experiment

1- a negative control group (-) that was left without a bacterial infection.
2- A positive control group (+) infected with Pseudomonas aeruginosa was left untreated for the duration of the experiment.

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3- A group infected with Pseudomonas aeruginosa was given 2% of vitamin C.
4- A group infected with Pseudomonas aeruginosa was given 4% of vitamin C.
5- A group infected with Pseudomonas aeruginosa bacteria was given 7 (mg/kg body weight) from standard chicken collagen.
6- A group infected with Pseudomonas aeruginosa bacteria was given 7 (mg/kg body weight) from standard chicken collagen with 2% of vitamin C.
7- A group infected with Pseudomonas aeruginosa bacteria was given 7 (mg/kg body weight) from standard chicken collagen with 4% of vitamin C.
8- A group infected with Pseudomonas aeruginosa bacteria was given 7 (mg/kg body weight) from laboratory chicken collagen.
9- A group infected with Pseudomonas aeruginosa bacteria was given 7 (mg/kg body weight) from laboratory chicken collagen with 2% vitamin C.
10- A group infected with Pseudomonas aeruginosa bacteria was given 7 (mg/kg body weight) from laboratory chicken collagen with 4% vitamin C.

Estimating the weights of some internal organs for the animals

Due to the importance of these internal organs in the metabolism process to benefit from food, the abdominal cavity for each rat was opened separately after anesthetizing the animals and taking blood samples from them. The internal organs of the rats (heart, brain, liver, kidney, spleen, and thyroid) were extracted using an anatomy kit. The internal organs were washed with normal saline, then dried on a filter paper and weighed in dry dishes using a sensitive scale.

Preparing white blood cells

The white blood cell count was calculated by diluting the blood sample with a ratio of 1: 2 using Turkey's Solution, after mixing the blood well with the solution left for 15 min, drops were then placed on the slide and the total white blood cell count was calculated in four squares according to the following formula (5):

$$\text{Total white blood cell count (cell.mm}^{-3}) = \frac{\text{number of calculated cells}}{10x20x4}$$

White Blood Cell Differential Count

The method described by (6) that summarized by making a swab for a blood sample, staining it with Leishman tincture, then examining it under a microscope, and calculating the types of white cells separately.

Estimating immunoglobulins

Immunoglobulins were estimated using the ELISA (Enzyme Linkage Immune System Assay) as mentioned in (18). where the number and solutions obtained from the French company Bio labo at the laboratory temperature for several minutes to ensure the evaporation of the water on its surface. The estimation was conducted by filling the holes in the device plate with a quantity of 5 ml of each blood sample and left for 15 minutes to complete the absorption process, then transferred to the incubator of the device at a temperature of 35 ºC for 72 hours, the readings were then recorded.

Statistical analysis

The experiment was conducted using completely randomized designs (CRD). The analysis of variance was performed using the General Linear Model within the ready-made statistical program (17). In the case of significant differences, Duncan's New Multiple Range Test (9) was used to determine the significant differences between the different averages at the probability level of 0.05.

3. RESULTS AND DISCUSSION

Effect on the relative weight of some internal organs

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Table (1) shows the effect of oral administration from two types (standard and laboratory chicken collagen) separately or with vitamin C on the relative weight of some organs in female rats induced infection with P. aeruginosa given for 28 days. The results showed that oral administration of animals at a concentration of 2 and 4% of vitamin C did not cause a significant change in the relative weights of the internal organs (heart, liver, kidneys, and thyroid gland), but it caused a significant decrease in the relative weights of the brain which amounted to (0.80 and 0. 76 g / 100 g body weight) compared to its relative weight in animals of the control group that amounted to (1.01 g / 100 g body weight). The same applies to the groups given to standard or laboratory chicken collagen when their values are compared with the positive control group. Likewise when oral administration of chicken collagen, whether standard or laboratory, the values of relative weight for the internal organs did not change significantly except in the case of the relative weight for the spleen in the group of animals given standard collagen with 2 and 4% of vitamin C, whose relative weight amounted to (0.50 and 0.47) compared to the relative weight of the spleen amounted to (0.62 g / 100 g body weight) in the animals of the negative control group. This is due to the effectiveness of collagen where it works as an antioxidant as well as its important role in improving the metabolism of fats, thus preventing the process of inflation that gets to the organs. Vitamin C is also the first line of defense against oxidative stress. These results agree with (15) who observed that there was no significant difference in the relative weight of the heart in the groups given to collagen and for 12 months in different concentrations, as well as for the relative weight of the brain, liver, kidneys, and thyroid gland. The (14) also mentioned that vitamin C has an important role in repairing damaged cells and it is also of great importance in detoxifying the mineral elements and toxins of bacteria and fungi. Collagen also has an important role in the work of the heart and blood vessels (12).

Table 1: Effectiveness of oral administration of collagen and vitamin C in the relative weight of internal organs in rats induced infection with P. aeruginosa after administration for 28 days.

The relative weight of internal organs for rats (g / 100 g body weight)							
Treatments	**Concentration**	**Heart**	**Brain**	**Liver**	**Kidney**	**Spleen**	**Thyroid**
Control (-)	0.0	0.37^{a}	0.73^{b}	3.81^{a}	0.74^{a}	0.62^{a}	0.07^{b}
Control (+)	0.0	0.28^{b}	1.01^{a}	3.84^{a}	0.76^{a}	0.39^{c}	0.36^{a}
Vitamin C.	2%	0.41^{a}	0.80^{b}	3.47^{a}	0.72^{a}	0.54^{b}	0.08^{b}
	4%	0.46^{a}	0.76^{b}	3.49^{a}	0.81^{a}	0.78^{a}	0.08^{b}
Standard chicken collagen	(7 mg / ml)	0.40^{a}	0.69^{b}	3.76^{a}	0.75^{a}	0.70^{a}	0.06^{b}
Standard Chicken Collagen + Vitamin C.	(7 mg / ml) + 2%	0.41^{a}	0.78^{b}	3.47^{a}	0.66^{a}	0.50^{b}	0.09^{b}
	(7 mg / ml) + 4%	0.44^{a}	0.81^{b}	3.35^{a}	0.67^{a}	0.47^{b}	0.09^{b}
Laboratory chicken collagen	(7 mg / ml)	0.44^{a}	0.80^{b}	3.67^{a}	0.86^{a}	0.64^{a}	0.07^{b}
Laboratory Collagen + Vitamin C.	(7 mg / ml) + 2%	0.45^{a}	0.74^{b}	3.44^{a}	0.64^{a}	0.60^{a}	0.07^{b}
	(7 mg / ml) + 4%	0.47^{a}	0.78^{b}	3.41^{a}	0.63^{a}	0.49^{b}	0.08^{b}

The different letters in one column indicate the significant differences between the averages at the probability level of 0.05

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Effect on the White Blood Cell Differential Count

Table (2) shows the effect of oral administration from two types (standard and laboratory chicken collagen) separately or with vitamin C on the White Blood Cell Differential Count in the blood of rats induced infection with P. aeruginosa given for 28 days. The results showed that infection of laboratory animals with P. aeruginosa type caused a significant ($P < 0.05$) increase in the total White Blood Cell Differential Count where their Counts in the negative and positive control groups amounted to (8.4 and 10.7 cells $\times 10^6$), and the increase was significant in the percentage of lymphocytes and the decrease was significant in the percentage of granulocytes compared to their Counts and percentages in the animals of the negative control group which amounted to (8.4 cells $\times 10^6$). The total White Blood Cell Differential Count decreased significantly in all used treatments and concentrations, where it amounted to (8.0 and 8.5) in groups treated with vitamin C at a concentration of (2 and 4%), respectively, compared to a positive control group that amounted to 10.7 cells $\times 10^6$), while it was 9.6 and 9.0 cells $\times 10^6$ in groups treated with standard chicken collagen and laboratory collagen, respectively. The table also showed a significant increase in the percentage and counts of lymphocytes in all used treatments and concentrations, where It amounted to (73.80 and 72.10%) in the groups treated with vitamin C at a concentration of (2 and 4%), respectively compared to the positive control group which amounted to 63.6, while amounted to (72.73 and 71.83%) in the groups treated with standard and laboratory chicken collagen, respectively. The total number of granulocytes decreased significantly in all used treatments and concentrations compared to the animals of the positive control group, which amounted to (34.3). These results agree with (1) who obtained in their results a significant increase in the total and differential numbers of blood cells in the groups infected with Salmonella bacteria and treating with collagen. The reason for the increase in the number of white cells in cases of inflammation is due to the presence of a substance in the plasma that stimulates the red bone marrow to increase the white cells. This substance is called the catalyst for the production of white cells Leucocytosis-inducing (3). A (4) has been reported that the functions of these cells are defensive and therefore increase in cases of inflammations and bacterial infections.

Table 2: Effectiveness of oral administration of collagen and vitamin C in the total and Differential White Blood Cell Count in rats induced infection with P. aeruginosa after administration for 28 days.

Treatments	**Concentration**	**Total white blood cell count**	**Lymphocytosis**	granulocytes
		(^{6}10×)	**%**	
Control (-)	0.0	8.4c	71.26 a	26.7b
Control (+)	0.0	10.7a	63.06 b	34.3a
Vitamin C.	2%	8.0c	73.80 a	24.1c
	4%	8.5c	72.10 a	25.8b
Standard chicken collagen	(7 mg / ml)	9.6b	72.63 a	25.9 b
Standard Chicken Collagen + Vitamin C.	(7 mg / ml) + 2%	8.8b	71.16 a	26.0 b
	(7 mg / ml) + 4%	7.2d	72.36 a	24.6 b
Laboratory chicken collagen	(7 mg / ml)	9.0b	71.83 a	24.5b
Laboratory Collagen + Vitamin C.	(7 mg / ml) + 2%	8.0c	72.73 a	23.9c
	(7 mg / ml) + 4%	7.7d	73.93 a	23.4c

The different letters in one column indicate the significant differences between the averages at the probability level of 0.05

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Effect on the immunoglobulin levels

Table (3) shows the effect of oral administration from two types (standard and laboratory chicken collagen) separately or with vitamin C on the levels of immunoglobulins (IgG, IgM, and IgA) in the blood of rats induced infection with P. aeruginosa given for 28 days. The results showed a significant decrease in the concentration of IgG in all oral administration treatments for rats compared to the positive control group infected with bacteria which amounted to (2777.8 mg/dl). The concentration of IgM decreased significantly in the case of animal groups administered orally by standard or laboratory collagen to one or with 2 and 4% concentration of vitamin C compared to their values in the control groups infected with bacteria or healthy which amounted to (152.4 and 197.3 mg / dL), respectively. It was observed that the concentration of IgA in the blood of adult female rats infected with P. aeruginosa and treated with standard or laboratory chicken collagen separately or with Vitamin C. at a concentration of 2 and 4% caused a significant decrease in all used treatments which amounted to (157.4, 147.9, 133.2, 158.2, 154.9) compared to its value in the positive control group which amounted to (392.2 mg / dL). The decomposed collagen has the potential to reduce inflammatory responses by producing cytokines via the glycine chloride channel gate (11). A study has revealed the role of collagen as an antimicrobial, where the defense mechanism includes the interaction between the peptide and the microbial cell membrane, which is a prerequisite for bacterial killing (19). A (2) mentioned that vitamin C has immune functions where it works to stimulate the inflammatory activity and the formation of antibodies, it also has an important role in the formation of immune bodies.

Table 3: Effectiveness of oral administration of collagen and vitamin C in the immunoglobulin levels in rats induced infection with P. aeruginosa after administration for 28 days.

Treatments	Concentration	IgG	IgM	IgA
		mg / dL		
Control (-)	0.0	1743.6f	197.3a	118.3d
Control (+)	0.0	2777.8 a	152.4e	192.2a
Vitamin C.	2%	2349.2c	160.8d	122.1d
	4%	2324.9c	188.2b	122.1d
Standard chicken collagen	(7 mg / ml)	2108.2d	189.4b	157.4b
Standard Chicken Collagen + Vitamin C.	(7 mg / ml) + 2%	1971.8d	174.3c	147.9b
	(7 mg / ml) + 4%	1901.1e	178.2c	133.2c
Laboratory chicken collagen	(7 mg / ml)	2497.4b	179.3c	158.2b
Laboratory Collagen + Vitamin C.	(7 mg / ml) + 2%	2234.5c	173.4c	154.9b
	(7 mg / ml) + 4%	2112.2d	174.3e	144.4b

The different letters in one column indicate the significant differences between the averages at the probability level of 0.05

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