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IN VIVO EFFECT OF *SPIRULINA PLATENSIS* AQUEOUS EXTRACT AND CHITOSAN NANO PARTIALS ON RATS WOUNDS, INDUCED BY *PSEUDOMONAS AERUGINOSA*

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Abstract

This study has documented the *In Vivo* activity for *Spirulina platensis* aqueous extract (SPAE) and Chitosan nanoparticles (CHNPs) towards *Pseudomonas aeruginosa* in wound infections of laboratory rats. SPAE at 75% and 200% or Ch-NPs 12.5% each one alone or when combined with Amoxicillin 25 µg were affected significantly on Biological parameter such as Urea, Creatinine, AST, ALT and immune cells that consist (White blood Cells, Lymphocyte, Neutrophil and Immunoglobulin A) of laboratory rats when they induced wounds with *P. aeruginosa* by decreased these biological parameters compared with infection treatments. The conclusion was showed that SPAE and CH-NPs has antibacterial and biological activity toward the bacterial isolate and biological parameter of rats and this activity increased significantly when mixture with Amoxicillin.

Key words **:** *Spirulina platensis*, Chitosan nanoparticles, *P. aeruginosa*, biological effect.

Introduction

The wound, is defined as disruption in the epithelial lining continuity of the skin, or mucosa resulting from the Physical or thermal damage. The wound is classified as a chronic and acute, according to the nature and duration of healing process (Robson *et al.*, 2001; Dhivya *et al.,* 2015). The pathogenic bacteria are those bacteria that have able to cause disease. Bacterial infection has a major effect on public health (Doron, 2017; Ghimire *et al.,* 2018). Natural products were used as medicines in the rural areas of several developing countries (Hosseinzadeh *et al.,* 2015). Spirulina now called (Arthrospira), produce a wide numbers of the bioactive compounds that has inherent medical advantages (Al-ghanayem, 2017). It is used as food supplements because their rich in nutritional content, with complete protein, lipids and carbohydrates. In addition, Spirulina has antioxidant and antimicrobial properties (Kore and Wakte, 2017). Chitosan is one of the most polymers used in field of Nano drug delivery system (Jesus *et al.,* 2020). (NPs), assumed an important role in the area of drug delivery. Despite the number of studies including NPs are growing over the last years, one of the most studied polymers in the Nano based drug delivery system field is chitosan. NPs of Chitosan have several applications in non- parenteral drugs delivery for treatment of gastrointestinal diseases, pulmonary diseases, gastrointestinal diseases, cancer, brain and ocular infections, in addition to their immune-improving and antimicrobial effect (Mohammed *et al.,*2017; Divya *et al.,*2017).

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Materials and Methods

Samples collection

62 wound samples were collected from wound infections of both sexes under sterile conditions by using sterile transport cotton swabs. 9 bacterial isolates were *P aeruginosa* that used in this experiment.

Bacterial isolates Identification

Colonies of bacterial isolates were identified, initially with the morphology appearance through notes the color, Consistency and the formed on MaCconkey's and Nutrient agar medium also according to hemolytic type on blood agar medium. (Alfred, 2005).

Microscopic examination

Microscopic examination were classified the bacterial

isolates according to the shapes or clusters noted under a microscope, also learn the positive or negative to gram stain Microscopic examination and according to their staining by gram stain, shape, size of cell arrangement. (Alfred, 2005).

Biochemical tests

Biochemical tests for p. aeruginosa were done according to Alfred, (2005) and Collee *et al.*,(1996).

Preparation of *Spirulina platensis* aqueous extract

Spirulina platensis was purchased from the company of DXN. The Spirulina aqueous extract solution 200% and 75% were prepared according to Singh *et al.,* (2014)

Preparation of Chitosan Nanoparticles(CHNPs) solution

CHNPs purchased from Nanoshel Company and CHNPs solution12.5% was prepared according Aliasghari *et al.,* (2016).

Laboratory Animal Configuration

Male white Rats (Albino Male rats) were used in this study equipped from Animal house of Veterinary Medicine College/ Tikrit University, and ensure it's free from disease by veterinarian supervised. The Rats were in ages (8-12 weeks) with 184.25g in rate weight. The Rats were kept in metallic cages with metallic cover and littered with sawdust. The cages sterilized by alcohol (99% ethanol). The laboratory condition was adjusted the ventilation and temperature at 23-25 ºC and photo period between 14 hours light –12hours dark. The animals were feed on main diet according to (Saleem *et al.,* 2011). The diet formula were consist on: Casein 158.5gm, 100gm sun flower oil, vitamins mixed 5gm, mineral salts 50gm, cellulose 50 gm, glucose 100gm and the starch at 536.5gm. These contents were mixed together and dissolved with 350-500 ml of distilled water to mix the diet then its formed to discs form with diameter 5*20mm before drying at laboratory temperature.

The dose infection preparation

Bacterial isolates from *S.aureus* and *Pseudomonas aeruginosa* were cultured in nutrient broth in sterilized glass test tubes and incubated at 37 ºC for 24 hrs. The turbidity of culture was compared with 0.5 McFarland solution tube, that used for bacterial suspension comparison that consist of 1.5×10^8 cell/ml that used to infected rat's wounds later.

Wounds initialization and infected with bacterial isolates suspension

The 54 white male rats were used in this experiment were separated to 12 groups. The scratches were done on rat's skin surface with sterilized lancet after septic with alcohol (ethanol 70%) and shaving the regions (Dale *et al.,* 2004). The wounds were contaminated with bacterial suspension that previous prepared except the control group. The wound infections were done using sterile swabs that contaminating with each *S.aureus* and *P.aeruginosa* suspension. Then the treatments were done used each of SPAE or ChNPs till to healing the wounds. Blood spacemen were taken after the wounds were healing to determine the blood parameters such as blood pictures, biochemical and immunological tests.

The treatments of rat's wounds were divided into three periods: First: Pre infection period (Control) Second: Infection period (Infections) Third: the treatment period.

First Treatment: Pre infection period

This period consists on animal groups, that wounds were make and non-infected, in pre-infection wounds period. and release of 4 rats' exposure to wounded without bacterial infection.

Second Treatment: infection period

They were consisting to separate the rats on two groups of infected with two bacterial isolates:

1. The rats were infected with *Staphylococcus aureus* bacteria.
2. The rats were infected with *Pseudomonas aeruginosa* bacteria.

Third Treatment: The treatments period

This period was including treated period for wounds infected Laboratory animals which are consist: The animals group infected with *P.aeruginosa* and treated with Amoxicillin antibiotic (25mcg)

1. The animals group infected with *P.aeruginosa* and treated with SPAE at 25mg/ml
2. The animals group infected with *P.aeruginosa* and treated with SPAE combination with Amoxicillin antibiotic (25mcg)
3. The animals group infected with *P.aeruginosa* and treated with ChNPs at 12.5mg/ml.
4. The animals group infected with *P.aeruginosa* and treated with ChNPs 12.5% combination with Amoxicillin antibiotic (25mcg)
5. The animals group infected with *S.aureus* and treated with Amoxicillin antibiotic (25mcg)
6. The animals group infected with *S.aureus* and treated with SPAE 25mg/ml.
7. The animals group infected with *S.aureus* and treated

with SPAE combination with Amoxicillin antibiotic (25mcg)

8. The animals group infected with *S.aureus* Treatment with ChNPs at 12.5mg/ml.
9. The animals group infected with *S.aureus* and treated with ChNPs at 12.5mg/ml combination with Amoxicillin antibiotic (25mcg)

The treated period was contained 10 days then the blood samples were given from ophthalmic vein of rats, samples are collected in two groups of blood tubes, one of them was contained on anti-coagulant substance (EDTA) for complete blood picture tests, while another group of tubes were without this substance, this group was centrifuged by Centrifuge apparatus at 3000rpm for 15min. to obtain the serum that kept in freeze at -20ºýC until the biochemical parameters assay tests will done later.

The blood assay

The blood components were measured by used Complete Blood Count(CBC) apparatus. The tubes that containing on (EDTA) used to done complete blood components picture. The blood components measured were consist on White blood cells (WBCs) and Red Blood Corpuscles (RBCs). The apparatus containing two chambers, first chamber for the purpose (WBCs) numbers accounting in addition to the blood hemoglobin measured by lysing Red Blood Corpuscles (RBCs) from added substance causing (RBCs) brake and release hemoglobin that measured by spectrophotometer, The second chamber was measured (RBCs) and blood platelets, The scientific basis of blood components measured by this apparatus depend on electric field saturated with isotone to charges equation and by fixed voltage of electric current passing through the tube when one of the blood components passes It generates partial resistance to the electrical current in the electric column.

Biochemical Test

Aspartate Aminotransferase (GOT/AST) Enzyme, Alanine Aminotransferase (ALT/GPT) Enzyme, Urea and Creatinine were measurements in serum of rats, the values were measured during infection,before and after treatment by using Kits for every test with UV-assay according to IFCC(International Fedration of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate by used mindray instrument.

Determination of immunoglobulin A (IgA) protein

Radial immunodiffusion plate method was used for IgA protein determined in serum of rats according to Good Laboratory Practice(GLP)(Mancini *et al.,*1965; Fahey and McKelvey,1965).

The statistically analysis

The experiment results was analyzed by Complete Randomized Design (CRD) according ready-made statistical program(SAS,2010) to determine the significance differences between differences mean at probability level (0.05) by using Duncan multi range test (Ducan, 1955).

Results and Discussion

The effects of SPAE or ChNPsý on Hb and RBCs of laboratory rats induced wounds with *P. aeruginosa*

The results in table 1 were observed the levels of Hb and RBCs of rats that induced wounds with *P. aeruginosa*. The results were investigate the effect of wound induced with *P. aeruginosa* in decreased significantly ($p<0.05$) of Hb which were became at 9.70g/dl and RBCs accounts at 5.90 (10^6/mm3) compared with control rats group (T_1) that at 12.06 g/dl for Hb and 8.43 (10^6/mm3) for RBCs respectively. The treatment of rats with 25mg of SPAE (T_5) or with 12.5 mg of ChNPsý (T_7) were effect on efficiency on the Hb and RBCs and become at 11.33 g/dl and 7.46 (10^6/mm3) and T_7 at 11.26 g/dl and 7.46 (10^6/mm3) respectively which were became at the same values of the parameters at control group.

The slightly acidic pH of skin surface considered favors to development of some bacteria, pH is a major role in skin's protective system, it prevent colonization with pathogenic microorganisms by creates a hostile environment (Surber *et al.,* 2018). *Pseudomonas aeruginosa* causing a serious complication infection wounds with a high rate of morbidity and mortality (Augustine *et al.,* 2015), It localized in the deepest wound region, the skin injuries constitute represent a gateway

Table 1: The effects of SPAE or ChNPsý on Hb and RBCs of laboratory rats induced wounds with *P.aeruginosa.*

Treatments	Hb (g/dl)	RBC (10^6/mm^3)
T_1	12.06±0.50	7.53±0.20
T_2	10.90±0.50	6.66±0.21
T_3	11.70±0.98	6.90±0.48
T_4	11.43±0.99	6.10±0.66
T_5	11.33±0.66	6.46±0.43
T_6	11.33±0.23	6.73±0.03
T_7	10.93±0.40	6.93±0.48
T_8	12.06±0.52	7.40±0.60

**Hb: Haemoglobin, RBCs: Red Blood Corpuscles, T_1: Control, T_2: Wounds without any treatment, T_3: Wounds infected with *P. aeruginosa,* T_4: Treatment with amoxicillin, T_5: Treatment with SPAE 25%,T_6: Treatment with SPAE 200% and AX, T_7: Treatment with chts NPs 12.5%, T_8: Treatment with combination ChNPs and AX.

for this pathogenic bacteria to causing establishment of chronic wounds by impair tissue integrity and keep the inflammatory phase by producing the virulence factors such as protease, lipase,urease, heamolysin in addition to bacterial motility that enables bacteria to adhere with epithelial cells of host, elastase, It's one of the kind of preotease that causes tissue necrosis, Lipases cause distraction the lipids of host cell membranes and hemolysin Which have associated with cytotoxicity and necrotoxicity of the host's cells also It form pores in the plasma membrane of erythrocytes (Nile *et al.,* 2015; Ribeiro *et al.,* 2020).

The effects of SPAE or ChNPsý on Urea and Creatinine of laboratory rats induced wounds by *P. aeruginosa*

The results in table 2 observed the effects of SPAE or ChNPs on Urea and Creatinine of laboratory rats induced wounds by *P. aeruginosa*. The results were showed that increased significantly ($p < 0.05$) of urea and creatinine and became at 54.53 mg/dl and 0.44 mg/dl respectively. The 0.1 ml swab of SPAE or ChNPs alone or each one with amoxicillin were caused efficacy the urea and creatinine parameters and become for T_5 at 35.30, 0.31mg/dl and at T_7 35.1, 0.29 mg/dl, also the T6 was became at 31.3, 0.22 mg/dl; T8 at 39.6, 0.33 mg/dl respectively when compared with T_3. The results indicate that all treatments were become equal or nearest to the value in control group T_1, except with the T_6 that was significantly decreased in Urea.

Chitosan nanoparticles had non–toxicity and biocompatibility; it can decrease mortality and morbidity of chronic kidney disease in humans (El-Marakby *et al.,* 2017). Chitosan used as a drug to promote renal function in the patients who have chronic renal failure. Also it caused reduction in urea and creatinine serum level; In addition it was non-harmful on the renal tissue in different types and concentrations (Williams *et al.,* 2015; Mustafa *et al.,* 2016). The results in table 2 were agreement with Rojas-Franco *et al.,* (2018) who detect the role of *Spirulina* and its components on mice glomerular function and creatinine level after intoxicated with mercury, they observed an improvement glomerular function of these mice and reduced the creatinine concentration in serum of mice. As well as, Spirulina decreased the serum creatinine, this due to phycocyanin which is one component of spirulina has a main role in reduce renal dysfunction and serum creatinine (Ferna´ndez-Rojas *et al.,* 2014).

The effects of SPAE or ChNPsý on AST and ALT of laboratory rats induced wounds by *P. aeruginosa*

The results in table 3 were showed the effects of 0.1 ml swab of SPAE or ChNPs on AST and ALT enzymes of laboratory rats induced wounds by *Ps.aeuginosa*. The results were showed significantly increased ($P<0.05$) the enzymes AST and ALT and became in T_3 at 183.3 and 95.1 IU/l respectively compared with values in control group (T_1) which appeared at 164.66 and71.66 IU/l. While, the SPAE or ChNPs were reduction the AST and ALT atin T_5 at 152.7 and 60.1 IU/L and in T_7 at 157.7, 61.3 IU/L, also AST and ALT were reduction in T_6 at 157.1, 62.6 IU/L and T_8 at 151.4, 64.6 IU/L respectively when SPAE or ChNPsý used with amoxicillin antibiotic compared with the wounds induced by *P. aeruginosa*. The T_4 was become at 164.1 and 68.86 IU/L respectively which was same parameters and was not significant different present compared with values for the same

Table 2: The effects of SPAE or ChNPsý on Urea and Creatinine of laboratory rats induced wounds by *P. aeruginosa*.

Treatments	Urea (mg/dl)	Creatinine (mg/dl)
T_1	40.5 ± 0.76bc	0.34 ± 0.01bc
T_2	44.5 ± 3.0b	0.38 ± 0.01b
T_3	54.5 ± 2.55a	0.44 ± 0.02a
T_4	38.3 ± 2.43bc	0.34 ± 0.02bc
T_5	35.3 ± 0.85cd	0.31 ± 0.01c
T_6	31.3 ± 1.82d	0.22 ± 0.01bc
T_7	35.1 ± 1.82bc	0.29 ± 0.008c
T_8	39.6 ±2.24bc	0.33 ± 0.008bc

The similar vertically letters means not Significant different in each column at probability at ($P \leq 0.05$).

T_1: Control, T_2: Wounds without any treatment, T_3: Wounds induced with *P. aeruginosa,* T_4: Treatment with amoxicillin, T_5: Treatment with SPAE 25%,T_6: Treatment with SPAE 200% and AX25µg, T_7: Treatment with Ch-NPs 12.5%, T_8: Treatment with combination Ch-NPs 12.5% and AX 25µg.

Table 3: The effects of SPAE or ChNPsý on AST and ALT of laboratory rats induced wounds by *P. aeruginosa*.

Treatments	AST (u/l)	ALT (u/l)
T_1	164.6 ± 1.85 bc	71.6 ± 3.28 b
T_2	168 ± 2.64 b	74 ± 2.88 b
T_3	183.3 ± 1.65 a	95.1 ± 1.76 a
T_4	164.1 ± 2.19 bc	68.8 ± 1.50 bc
T_5	152.6 ± 3.01 d	60.9 ± 1.91 d
T_6	157.9 ± 4.22 cd	62.6 ± 1.55 cd
T_7	157.7 ± 1.50 cd	61.2 ± 2.11 d
T_8	151.4 ± 2.11 d	64.6 ± 0.89 cd

The similar vertically letters means not Significant different in each column at probability at ($P \leq 0.05$).

T_1: Control, T_2: Wounds without any treatment, T_3: Wounds induced with *P. aeruginosa,* T_4: Treatment with amoxicillin, T_5: Treatment with SPAE 25%, T_6: Treatment with SPAE200% and AX25µg, T_7: Treatment with Ch-NPs 12.5%, T_8: Treatment with combination Ch-NPs 12.5% and AX 25µg.

parameters in control group T_1.

The results were agreed with Wagner *et al.,* (2008) and Luczkiewicz *et al.,* (2008) who reported that Pseudomonas bacteria can to cause changes in ALT and AST of serum, because its ability to cellular degradation of liver or heart muscle, also they suggested that, the increased production in the AST and ALT levels activity represent an indicator to cell necrosis of many tissues and damaged organs. *The Ps.aeruginosa* is mainly production of many extracellular virulence factors such as pyoverdin, pyocyanin, elastase, phospholipase, protease and hemolysin that can damage a large of tissue components like, connective tissue of proteinaceous elements in addition to responsible for inflammation that leading to organ failure (Pramodhini *et al.,* 2016; Luczkiewicz *et al.,* 2015). The virulence factors in a high amount are released in the blood circulation, and since the liver contribute in clearing the body from them and it remains the first target to be activated by virulence factors that releasing active amount of inflammatory molecules and leading to liver injury (El-Gendy *et al.,* 2017 ; Kumar *et al.,* 2014). *Spurulina* treatment led to decrease in AST and ALT because of antioxidant property of *Spurulina* that drastically attenuated the damage of liver's tissue in addition to acts as liver detoxifier and as catalyst of essential elements absorption (Hyo-Jin *et al.,* 2006). The *Spirulina*, is rich in beta carotene (β-carotene) and the bioavailability consider as good as the pure selenium, vitamin E, vitamin C in addition to β-carotene also the SPAE effective against the free radical induced peroxidation of lipid which in turn may cause cellular transformation (Pal *et al.,* 2010). Also ChNPsý have a role in protective against oxidative stress and hepatotoxicity in liver tissues. Its induce improvement in cytokines, antioxidant enzymes and oxidative stress markers (Wen *et al.,* 2013; Abdel-Wahhab *et al.,* 2017). The results were agreed with (Subhapradha *et al.,* 2013) who conducted that β-Chitosan normalize plasma ALT and AST levels in (CCl_4-treated rats) and this indicate to that β- chitosan maybe prevent intracellular enzymes leakage into the blood by keep the cell membrane stabilize. Consequently the effect of Chitosan on overall hepatoprotective is probably due to free radicals counteraction through its ability to inhibit accumulation of lipid by its antilipidemic property and/or anti-oxidant nature (Sivakumar *et al.,* 2007; Ramasamy *et al.,* 2014).

The effects of SPAE **or ChNPsý on some immune cells of laboratory rats induced wounds by *P. aeruginosa***

Table 4 was showed the effects of 0.1 ml of SPAE or ChNPsý on some immune cells of laboratory rats induced wounds by *P. aeruginosa*. The treatment of rat wounds group (T_3) with *P. aeruginosa* were effects on increased significantly ($p< 0.05$) the WBCs at 23.2 $\times 10^3$/ mm^3, Lymphocytes at 68.9% and Neutrophil at 28.7% compared with control rats group (T_1) which (17.7) $\times 10^3$/ mm^3, 10.9 %, 56.2% and 20.3% respectively. The SPAE or ChNPs alone or each one with amoxicillin were caused efficacy the all immune cells under study compared with T_3 and they become at same parameter of control group except lymphocytes at T_6 47.1% which were decreased significantly compared with T_1, T_4 there were not observed significant different present in WBCs at 16.1 $\times 10^3/mm^3$, Lymphocytes at 53.1% and Neutrophiles at 18.5% respectively compared with control group T_1 for each one, also with T_5, T_6, T_7 and T_8.

Skin provides protection against constantly environmental changes and the pathogenic intrusion and the breach of this barrier induces fine coordination between the cells and molecular factors to reestablish, maintain the integrity of the skin structure and function (Gonzales and Fuchs, 2017). The wound healing process includes tightly orchestrated and largely interfering phases (Hemostasis, Inflammation, Proliferation, and Remodeling). The wound's infiltration occurs by immune cells such as monocytes and lymphocytes in the inflammatory phase. Also this barrier considered a rich and diverse in microbiota that includes on pathogenic and opportunistic microorganisms which could be useful for the skin microenvironment (Palta *et al.,* 2014; Nguyen and Soulika, 2019), so the skin naturally acts as protective barrier towards such microorganisms that trying seize new areas on the host organism, The immune system therefore acts as adeterrent against this opportunistic microbes, so that in the aggression of microbes case the immune system intensifies its response (Cogen *et al.,* 2008 ; Grice and Segre, 2011). For this reason that's why high immune cell ratios can be observed in wounds treatment.

Bacterial products induce the innate immune system, a bacterial cell makes various proteins that do not likes those made by cell of animal and thus play a main role in the beginning of inflammation (MacLeod and Mansbridge, 2016).

The effects of SPAE **or ChNPsý on IgA concentration of laboratory rats induced wounds by *P. aeruginosa***

The results in table 5 were illustrated the effects of SPAE or ChNPs on IgA concentration of laboratory rats induced wounds by *p. aeruginosa*. The results were

Table 4: The effects of SPAE or ChNPsý on some immune cells of laboratory rats induced wounds by *P. aeruginosa*.

Treatments	WBCs (10^3/mm^3)	Lym. %	Net. %
T_1	17.7 ± 0.91 b	56.2 ± 0.39 b	20.3 ± 0.33 bc
T_2	20.5 ± 2.69 ab	63.4 ± 1.99 a	22.6 ± 0.66 b
T_3	23.2 ± 0.58 a	68.9 ± 2.25 a	28.7 ± 3 a
T_4	16.1 ± 1.41 b	53.1 ± 2.56 bc	18.5 ± 0.29 c
T_5	16.5 ± 0.74 b	53.6 ± 1.30 bc	18.2 ± 1.40 bc
T_6	16.4 ± 1.83 b	47.1 ± 3.58 c	15.5 ± 0.73 bc
T_7	16.2 ± 0.98 b	55.8 ± 1.29 b	18.4 ± 1.70 bc
T_8	15.7 ± 1.28 b	52.9 ± 1.48 bc	18.2 ± 1.56 bc

The similar vertically letters means not Significant different in each column at probability at ($P \leq 0.05$).

T_1: Control, T_2: Wounds without any treatment, T_3: Wounds induced with *P. aeruginosa,* T_4: Treatment with amoxicillin, T_5: Treatment with SPAE 25%, T_6: Treatment with SPAE 200% and AX 25µg, T_7: Treatment with Ch-NPs 12.5%, T8: Treatment with combination Ch-NPs 12.5% and AX 25µg.

Table 5: The effects of SPAE or ChNPsý on IgA concentration of laboratory rats induced wounds by *P. aeruginosa*.

Treatments	IgA(µg/ml)
T_1	503 ± 7.23ab
T_2	513 ± 8.81ab
T_3	525 ± 8.54a
T_4	484 ± 8.08 bc
T_5	458 ± 13.56c
T_6	452 ± 12.70c
T_7	454 ± 7.21c
T_8	455 ± 7.62c

The similar vertically letters means not Significant different in each column at probability at ($P \leq 0.05$). T_1: Control, T_2: Wounds without any treatment, T_3: Wounds induced with *P. aeruginosa,* T_4: Treatment with amoxicillin, T_5: Treatment with SPAE 25%,T_6: Treatment with SPAE 200% and AX25µg, T_7: Treatment with Ch-NPs 12.5%, T_8: Treatment with combination Ch-NPs 12.5% and AX 25µg.

showed significantly increased($P< 0.05$) at T_3 at 525 compared with rats control group (T1) which was at 503. The extract of *Spirulina platensis* or ChNPs alone or each one with amoxicillin were caused efficacy the urea and creatinine and they become at T_5 at (458),T_6 at (452) and at T_7 (454) respectively compared with T_3, and they were significantly decreased($P<0.05$) compared with T_1. The treatment of rat wounds which induced with *P. aeruginosa* and treated with amoxicillin 25µg alone (T_4) at 496 not observed significant different at ($P< 0.05$) in IgA concentration compared with T_1, T_5, T_6, T_7 and T8respectively.

The skin consist on complex network of immune cells that resident to the tissue and it crucial for host defense and tissue homeostasis, such as keratinocytes, dendritic cells, monocytes, fibroblasts, and macrophagesin addition to sweat and sebaceous glands (Niyonsaba, *et al.,* 2017). B cells can represent as powerful modulators of tissues regeneration and it interact with innate cells such as dendritic cells, So the B-cell induced to secrete IgA to damaged tissues to engaged in process of wound healing (Hoffman *et al.,* 2016; Nguyen and Soulika, 2019; Suzuki *et al.,* 2010). IgA increased with infection with *P. aeruginosa*, this explained by several studies such as study of (Curran *et al.,* 2018) who observed that *P. aeruginosa* induce IgA production Because it enable to evading innate and adaptive immune responses. When bacterial infections are occur, specific immunoglobulin will bind to the bacterial surface and induce killing the bacteria. Also antibodies specific for this microorganisms, antibodies to tissue antigens also reach wound sites. Antibodies are secreted from B-cells which might migrate to wounded site(Suzuki *et al.,* 2010). *P. aeruginosa* able to produce many virulence factors such as haemolysins and proteases that attack IgA (Jimenez *et al.,* 2012). Exoenzymes responsible for host tissue damage by damage normal structure of cytoskeletal and cleavage (IgA) to development of chronic infections (Chatterjee *et al.,* 2016). This lead to the production of mediators, also recruitment of additional immune cells subsets (Lovewell *et al.,* 2014). Also amoxicillin caused in elevated in IgA and this may be related to bacterial resistant (*P. aeruginosa*) to this antibiotics. *Spirulina* extract 25% was reduced IgA in rats serum, and this disagreed with (Matufi *et al.,* 2020) who suggested that *Spirulina* responsible on buildup of both the cellular and humeral of the immune system, and it possess anti-allergic Characteristics by inducing IgA antibody. increased IgA concentration observed in IgA this may related to the effect of *Spirulina* on bacteria, Because it is known for its antibacterial properties because it contains effective phycocyanin against bacteria like *P. aeruginosa* as it mentioned (Nuhu, 2013). As it known the ChNPs effectiveness towards gram positive and gram negative bacteria, and this antibacterial activity of increase when it loaded with antibiotics (Ibrahim *et al.,* 2015). Although of antibacterial activity *In vitro* experiment of amoxicillin but it remains the most common antibiotic caused allergy it elevated Immunoglobulin E (IgE), so that the IgA will secreted to prevent the allergic inflammatory reactions development to environmental allergens (Balzar *et al.,* 2006; Pastorello *et al.,* 2015; Abrams and Khan, 2018).

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