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PHYSIOLOGICAL CHANGES FOR RABBITS INFECTED WITH *S. AUREUS* BY USING PHARMACEUTICAL COMPOUNDS AND PLANT EXTRACTS AS TREATMENTS

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ABSTRACT

S. aureus is an opportunistic pathogen that causes a wide spectrum of different diseases in humans and animals. *S. aureus* infects the skin of rabbits, causing irritation of some dermal tissue suppurative dermatitis and invades subcutaneous tissues, causing different severe diseases and different physiological changes. The aim of this study is to update knowledge and information on rabbit Staphylococcosis by concentrating mostly on the various symptoms and studying the physiological changes under the effect of pharmaceuticals and extracts of herbal plants as treatments for infected rabbits. Isoteritonin was used as a pharmaceutical treatment, while extracts of *Beta vulgaris* L. (beetroot) and Kaempferia pandurate (Chinese ginger) were used as an herbal treatment.

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Introduction

Bacterial skin infections are common in most countries [1]. The skin disease in different countries is ranged from 10-80%. Low levels of hygiene, which include unclean water, overcrowded populations, and hot and humid climates, are acting as etiologic factors for bacterial skin infections [2]. *Staphylococcus aureus* is a pathogenic bacteria that causes a wide variety of infections in humans [3]. *Staphylococcus aureus* is a harmful pathogen, capable of persisting and multiplying under various environmental conditions. It causes different diseases for both humans and animals. *S. aureus* is the major agent associated with nosocomial infections in men [3]. Untreated skin infections caused by *S. aureus* can extend to other parts of the body, causing different diseases, such as arthritis, osteomyelitis, endocarditis, pneumonia, and toxic shock syndrome [4].

S. aureus can asymptomatically colonize human skin, causing skin diseases (boils, impetigo). Staphylococcal infections in animals lead to economic losses in the livestock industry worldwide. *S. aureus* can infect the skin of rabbits superficially and also invade subcutaneous tissues [5], causing different pathologies, including mastitis, pododermatitis, and multisystemic abscessation [3]. *S. aureus* has different virulence factors, which enable it to cause disease of the skin, the eye, the respiratory system, and other septicemia in rabbits [1].

Isotretinoin, also known as Roaccutane, Accutane, and xeractan, is a derivative of vitamin A [1]. This drug has an important role in the treatment of acne nodules and inflammatory lesions [6]. The activity of isotretinoin is due to its ability to attach to and stimulate the different nuclear receptors of the acid retinoic acid; stimulation of such receptors acts as catalysts that induce cell differentiation and programmed death, thereby reducing the activity of tumors and also boosting immune responses as well as anti-inflammatory agents [6]. Isotretinoin poses different side effects associated with its use in the treatment of acne, such as increased risk of skin infections, functional liver disorders, and bowel inflammation [6]. Isotretinoin as a treatment for pregnant women leads to severe problems in the fetal nervous system [7]. Isotretinoin has toxic effects on kidney and liver functions with cellular and tissue damage [7]. Long-term use of high doses of isotretinoin results in patients developing

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autoimmune hepatitis, which is accompanied by a significant rise in their liver function test results [7]. Different symptoms of indigestion, abdominal pain, and nausea were recorded, while constipation and anal hemorrhage have been the most prevalent ones [7].

There are different essential plants that have traditionally been used to treat different skin infectious diseases. *Kaempferia pandurata* belongs to the family Zingiberaceae is the most important medicinal plant that has potent antibacterial activity and grows in different parts of Asia [8]. Fresh rhizomes of ginger are good treatments for inflammatory diseases like dermatitis, wounds, swelling, cold, dry cough, tooth, dental caries, gum disease, inflammation of women's uterus, vaginal infection, diarrhea, irritation of the colon, stomach bloating, difficult urination in children, dysentery, and as a diuretic [9]. A low concentration of ginger rhizome extract had excellent antibacterial activity against *S. aureus* [9]. This plant contains different essential oils and flavonoid compounds that have broad biological activities. Prenylated flavonoids from *Kaempferia pandurate*, a panduratin derivative, have shown a variety of biological activities, including potent antimicrobial activity, anti-cancer activity, and anti-inflammatory activity [10].

Red beetroot (*Beta vulgaris* L.) is considered a functional food that promotes health and is used as a natural medicine by Roman people [11]. Beetroot contains high amounts of different substances, including phenols, betalains, carotenoids, B-vitamins (B1, B2, B3, B6, and B12) fibers, folate-minerals, as well as sugars with low energetical value, and inorganic nitrate. [11]. All parts of this plant have different medicinal uses, such as antimicrobial, anti-inflammatory, anti-oxidant, anti-depressant, diuretic, expectorant, and carminative, hepatoprotective [12]. Red beetroot extract is used for the treatment of some chronic diseases, including diabetes, cardiovascular disease, cerebrovascular disease, cancer, and chronic respiratory disease [12].

Ingestion of beetroot has different advantageous physiological impacts, which could translate to improved clinical results for a variety of diseases, including elevated blood pressure, atherosclerosis, T2D, and dementia [11]. Its physiological effects are due to its contents of betalains [12]. Members of the betalain family include betaxanthin and betacyanin pigments, which exhibit strong anti-inflammatory and anti-oxidant properties in both in vitro and many in vivo models of animals [13]. Several lines of evidence have shown that betalains might reduce the risk of some cancers, immune dysfunction, cardiovascular and cerebrovascular diseases, arthritis, liver and kidney damage [13].

Because of the significance of isotretinoin in treating different skin diseases, particularly acne, it is very important to study the side effects of isotretinoin. Thus, this research sought to assess the side effects of isotretinoin on blood values and liver and kidney functions in rabbits infected by *Staphylococcus aureus* as the causative agent of acene and study the effect of medicinal plants (*Beta vulgaris* L. and *Kaempferia pandurate*) as treatments.

Material and Methods

Experimental Animals

Twenty rabbits (white Newzeland) of 1–1.5 Kg in weight were obtained from Sajer City and used in an experiment to examine the pathogenesis and harmful effects of *S. aureus* and study the effects of isotretinoin and extracts of two medicinal plants as a treatment for infected rabbits **(Figure 1)**.

Bacterial Strain

S. aureus was obtained from a microbiological lab at Al-Dawadmi General Hospital. One colony from a blood agar medium was inoculated into 10 milliliters of trypticase-soy broth to prepare the bacterial inoculation. The cultures have been centrifuged at 3000 rpm for 30 minutes before being decanted. A quantity of sterile normal saline was used to resuspend the *S. aureus* sediment. We prepared serial dilutions of each inoculum in normal saline. Bacterial colonies were counted by incubating 0.1 ml sections of bacterial suspensions on nutrient agar for 24 hours and then counting the number of colonies [5]. This experiment employed a measured volume of resuspended cells with a dosage of 1 x 108 CFU/ml [14].

Preparation of Pharmaceutical and Herbal Treatments

Preparation of Isotretinoin (Xeractan)

The Xeractan utilized in this investigation was acquired from the pharmacy in Sajer in the form of capsules. Every capsule had a 20 mg/60 kg of rabbit weight active dosage. The content of five capsules (100 mg) was mixed with drops of glycerol and then gradually added to 200 ml of distilled water with stirring.

Figure 1. Symptoms of infection with *Staphylococcus aureus*

Preparation of Beetroot Extract

(*Beta vulgaris* L.) was obtained from the market in Sajer City, washed in running water, cut into small pieces, and prepared using a domestic food processor. After juice separation, the sample of the obtained beetroot was stored at -20 °C until used as treatment. 50 ml of beetroot extract mixed with 200 ml distilled water **(Figure 2)**.

Figure 2. Preparation of Beetroot extract

Preparation of Chinese Ginger Extract

The dried Rhizome of *Kaempferia pandurate* (Chinese ginger) was obtained from the market in Al-Dawadmi Governorate. The dried rhizome was grounded, and 25g of ground rhizome was dissolved in 200 ml of distilled water **(Figure 3)**.

Experimental Design

The experimental rabbits were kept in the cage under normal conditions for 1 week for adaptation, and following that, they were split randomly into three infected groups (n=5 for every group) with a dose of $1x10^8$ CFU/ml of *S. aureus* and a control group (n=5) was administered sterile distilled water in the same way. All infected rabbits were held for seven days (the observation period), and everyday examinations were made for clinical symptoms, death rates, and inflamed lesions on dead animals until the observation period's end. The rabbits were treated after seven days of infection. The treated groups include the (I/D) group, which was treated with isotretinoin only; the (I/B) group, which was treated with isotretinoin and beetroot extract; and the (I/C) group, which was treated with Chinese ginger only. The period of treatment was seven days. At the end of the treatment period, 3 ml of blood samples from the marginal ear vein of each rabbit were taken from the control group (A) and three (infected/treated) groups for Hematological and Serum biochemical analysis.

Figure 3. Preparation of Chinese Ginger extract

The Blood Samples

Three ml of blood samples with anticoagulant were taken in (7) days to evaluate some blood parameters, including (RBCs) and white (WBCs) blood cell count, hemoglobin content (Hb), hematocrit (Ht) MCV, MCH, MCHC, and platelet. The serum of blood samples was taken to detect triglyceride, low-density lipoprotein, high-density lipoprotein, and cholesterol calorimetrically by using commercial kits (Biosystems S.A. Costa Brava, Barcelona, Spain). The calorimetric method was used to determine the transaminase enzyme activity of the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Aboelfadl *et al*. used commercial kits from Sclavo Diagnostics Company (Kite Italia SPA) to assess serum creatinine and urea using a colorimetric approach [15].

Statistical Analysis

The results of this study have been presented as mean± SEM. One analysis of variance (ANOVA) was used to determine the group variation. At $p<0.05$, statistical significance analysis was taken into account. Statistical analysis was done using SPSS [15].

Results and Discussion

In the present study, after a week of injecting the rabbits with *Staphylococcus aureus*, some clinical signs were observed in rabbits, such as loss of appetite, zigzag fur, decreased activity, the tendency of rabbits to escape at the corner of the cage, the emergence of clear red infections, rabbit fur falling out, especially in the injection area, nose and ear, and the appearance of purulent abscesses on rabbit skin and red eyes. These results nearly resembled those recorded by a previous study; different clinical signs were noticed, like ruffled fur, depression, loss of appetite, disinclination to move, a tendency to isolate themselves in a cage corner, mucopurulent conjunctivitis, coughing up pus, and diarrhea. At the inoculation site and in subcutaneous tissue, abscesses can occasionally be palpable. In the final stages, diseased animals suffered from emaciation, which was followed by death [16].

The body weight of each rabbit in the control group and the other three treated groups was detected during the experimental periods. There are no significant variations ($p<0.05$) in body weight in the infected group during the experimental periods compared with the control group **Table 1**. According to a previous study, a significant decrease in the body weight of rabbits infected with *S. aureus* in comparison to a control group was detected. This has been caused by a systemic response to the bacterial infection and its toxins on enteral organs, which was proven by positive bacterial isolation and the impact of the bacterial toxin on the digestive system, which affects food absorption from the intestine [17].

Table 1. Comparison of weights at the beginning and at the end of the experiment between control and three infected groups of rabbits (I/D, I/B, and I/c), which were treated with (xeractan, xeractan with beetroot, and Chinese ginger)

Group	1st Weight	2^{se} weight
A	1.220±83.66	1.146±67.68
I/D	1.196±71.274	1.095±10
I/B	1.160±108.39	1085±143.2
I/C	1.263±110.8	1.120±135.09

Values are expressed as means ± SE for female New Zealand rabbits groups (I/D, I/B, I/C). Data are significant at $p<0.05$. *: significantly different compared to group A

A: Control group I/B: Treated group by xeractan with beetroot, I/C: Treated group by Chinese ginger, I/ D: Treated group by xeractan only

The experiment animals showed different clinical signs after injection; it is considered a normal reaction to the irritant-injected pathogenic bacteria. On the other hand, the death of animals occurs due to the lethal toxin effect of *Staphylococcus aureus,* which kills within (3-24 h). This indicates the disturbance happens in blood, liver, and kidney functions in infected rabbits. The typical inflammation response to *Staphylococcus aureus* infection is characterized by the accumulation of inflammatory cells at a different part of the organ mainly (macrophage, neutrophils, and lymphocytes), which leads to abscessation inflammation at different organs such as the liver, kidney, heart, lung, trachea, and brain with degeneration changes [18].

Staphylococcus aureus infection affects liver and blood functions; a previous study was recorded after analyzing blood samples and measuring the level of haptoglobin, a protein found in blood plasma and secreted by the liver at normal rates as long as the liver is in good condition. The function of this protein is to link with free hemoglobin and form a compound that works to stabilize the percentage of iron in the blood and thus protects rabbits from anemia, but with the analysis of blood samples of rabbits, after injecting them with bacteria, found that the level of this globin is very low in the blood plasma, which indicates the presence of a major defect in the liver organ that eventually leads to anemia [19]. Infection with *Staphylococcus aureus* bacteria leads to severe inflammation in liver cells with damage necrosis of these cells, resulting in hepatic hemorrhage and also increased rate of infiltration and the formation of antibodies to resist and attack bacteria with the presence of congestion places, pus foci, and blood pools on the liver and a deficiency of total protein in the liver [18]. The infection of rabbits with *Staphylococcus aureus* bacteria leads to the occurrence of swelling in the renal tubules with the occurrence of bleeding in them and the presence of necrosis and pus foci in the renal cells and the occurrence of inflammation and infiltration as a result of the production of macrophages to attack the bacteria with the occurrence of interstitial bleeding in the renal cortex and the occurrence of a deficiency in the kidney's protein [19].

Isotretinoin is an effective oral treatment used to treat severe cystic acne and acne that does not respond to topical treatment. The toxicity of isotretinoin on organs, especially the liver and kidney, was recorded in previous studies [19]. Several studies have been conducted on herbal medicinal plants to find out the extent of their effective effect on skin infections and acne, and they were used as an alternative to pharmaceutical drugs. Medicinal plants are very effective in eliminating some of the side effects caused by chemical drugs [19]. In this study, Chinese ginger and beetroot extracts were used to treat the harmful effects of *Staphylococcus* and the side effects of isotretinoin.

Physiological Analysis

Hematological and Biochemical Analysis

Hematological

The statistical analysis of blood parameters (RBCs, WBCs, HB, Ht, MCV, MCH, MCHC) for blood samples was collected from the control group (A) and three infected groups with *Staphylococcus aureus* treated by xeractan with beetroot (I/B group), Chinese ginger (I/C group) and by xeractan (isotretinoin) (I/D group), respectively were detected in this study.

Results showed a difference in values of blood parameters .There was no considerable variation in blood parameters (WBCs, Ht) between healthy rabbits (control group) and three infected groups treated by xeractan with beetroot (I/B) group, Chinese ginger (I/C group) and the infected group treated with xeractan only (I/D group). The values of the MCV parameter decrease in all groups (I/B, I/C, I/D) compared to the control group (A). There was a significant increase in RBC counts in groups (I/B, I/C, and I/D) ($P< 0.05$) compared to the control group. The values of MCV decreased in all groups (I/B, I/C, I/D) compared to the control group (A). There has been a small reduction in HB and MCHC values in all treated groups in comparison to the control group. There was a significant decrease in MCH values ($P< 0.05$) in all treated groups with the control group. The values of platelets were high in (I/D & I/B) and low in groups I/ C compared to the control group (A) **(Table 2)**.

In a similar study whereas, the experimental rabbits were divided into four groups. Group (1) was control, groups (2, 3, and 4) referred to the infected rabbits with *Staphylococcus aureus*. The blood samples were taken from infected groups at different times (1, 15, 30 days after infection) from groups (2, 3, and 4, respectively). Also, blood samples were taken from the control group at the same periods. The statistical analysis showed a significant rise in WBC counts ($P<0.05$) among the two infected groups (3, 4) and the control group (1), as well as among groups (2) and (4), with no significant differences among the groups (3 and 4). A significant increase in MCV was detected between the treated groups (3, 4) and the control, while non-significant differences were recorded between two treated groups (3 and 4). Additionally, MCH found significant differences ($P<0.05$) among group (3) and the control group, as well as among group (3) and group (4) groups, but not among group (2) and the other groups. When comparing the treated group (4) to other groups, MCHC found significant differences ($P<0.05$) [20].

The previous study revealed the effect of using isotretinoin as a treatment on blood values in some cases of severe acne. The results showed the median Hb level was 14.07 ± 1.75 (g/dL) before the treatment and 14. 20 ± 60 (g/dL) after the treatment; the median Hct was 42.34 ± 4.31 (%) before the treatment and 42.83 ± 4.18 (%) after the treatment; and the median WBC count was 7488 ± 1969 (c/mL) before the treatment and was 7286 ± 2208 (c/mL) after the treatment. No statistically significant difference has been noticed for Hb (P = 0.352), Hct (P = 0.130), and WBC counts (P = 0.300). The median PLT count was 276231 ± 57683/μL before the treatment and 264351 ± 60680/μL after the treatment; the median McV count was 10.12 ± 1.47 (fl) before the treatment and was 9.27 ± 1.54 (fl) after the treatment. Following the therapy, the levels of PLT and McV both significantly dropped ($P = 0.020$ and $P < 0.001$, respectively). These results were nearly close to the results of our study, except the difference was detected in McV and PLT counts [21].

Table 2. Comparison of Blood Cells (WBCs, RBCs, Hb, Ht, MCV, MCH, MCHC, PLATELET) between control and three infected groups of rabbits (I/D, I/B, and I/c) treated by (xeractan, xeractan with beetroot and Chinese ginger)

Groups	WBCs $10^3/mm^3$	RBCs $10^6/mm^3$	Hb g/dl	Ht %	MCV $10^3/mm^3$	MCH $10^6/mm^3$	MCHC g/dl	Platelet %
A	8.9±1.93	4.54±0.76	13.56±1.83	36.6±17.83	75.88±9.94	32.32±2.35	33.78±2.64	279.8±1117.3
I/D	10.1±4.3	6.05 ±1.24	10.29±2.29	36.27 ±6.72	60.73 ±0.60	16.96 ±1.05*	28.27±2.34	373.5 ±313.2
I/B	7.91±1.29	6.48±0.245*	10.86±0.42*	37.75±2.27	58.29±1.28*	16.75±0.04*	28.8±0.65*	294.75±114.2
I/C	12.0±4.1	5.95 ±0.13*	12.51±1.33	38.38±2.24	64.14±2.42	17.43 ±4.56*	30.32±0.49	262.2±94.9

Values are expressed as means ± SE for rabbit groups (I/D, I/B, I/C). Data are significant at $p<0.05$.
*: significantly different compared to group A

Only a few investigations have shown higher PLT counts caused by isotretinoin. The results of the study by Karadag *et al*. showed that the platelet count was only modestly increased after receiving isotretinoin therapy, with no noticeable changes in the concentrations of WBC, Hb, or Htc. According to another study, 10% of the patients (n = 254) experienced elevated platelet counts [22]. PLTs are considered significant inflammatory mediators. Numerous investigations have shown that PLTs rise during inflammation and degranulate in infectious settings [22]. Isotretinoin acts as an anti-inflammatory, so it may reduce PLT and MPV either through its anti-inflammatory impact or by suppressing the bone marrow, as shown with the use of various chemotherapeutics [23].

In this study, the values of different parameters (WBCs, RBCs, Hb, MCV, MCH, MCHC, and PLATELET) which were recorded in the group (I/B) were nearly close to the values which were recorded in group (A) that indicate the beneficial effect of beetroot extract when mixed with xeractan in the treatment of infected rabbits; beetroot extract may decrease the side effects of using xeractan only. In a previous study, Al-Aboud determined the effect of beetroot intake on some blood parameters in a group of female volunteers; the results revealed that the beetroot extract intake increased the level of Hb, MCV, and ferritin. Extract of *B.vulgaris* as treatment leads to a significant increase in Hb concentration, RBC counts, packed cell volume, total lymphocyte count, and MCV [24]. So, it is suggested that the extract of beetroot is a good treatment for anemia because it includes folic acid, which is necessary for the production of red blood cells, the increase of hemoglobin, and the improvement of oxygen capacity, which enhances the flow of blood and has a significant role in the development of the fetal brain [25].

The value of WBC in group (I/C), which was treated with an extract of Kaempferia *pandurate*, was very high when compared to the value in the control group, that is due to the extract of *Kaempferia pandurate* containing many active compounds that function as immunomodulators by increasing the body's immunity through their antimicrobial properties. The extract contains pandaduratin A and 4-hydroxypandaduratin, which act as strong antibacterial agents against gram +ve and gram -ve bacteria [25].

Table 3 revealed the detected values of liver enzymes and lipid levels in different treated groups (I/B, I/C, and I/D) in comparison to the control group (A). There was a highly significant difference in LDL in all groups. There was a highly significant difference in cholesterol in groups (I/B&I/C) ($P< 0.05$). AST, ALT, and HDL recorded a highly significant difference in group (I/C) only ($P< 0.05$). There were no significant triglyceride values in all treated groups. AST values were high in all infected groups in comparison to the (A) group, and the highest AST value was recorded in group I/ C (169.4±45.2). The ALT values were high in group I/C and in group, I/ B (160.5 ±32.03 and 78.9±31.5, respectively) compared to the control group (70.7±18.5), while the ALT value in the group (I/D) was very low (57.2 ±16.09) in comparison to the value of control group (A). LDL values in all infected groups were high in comparison to group (A), with the highest value recorded by group (I/C) (117±59.9). The highest HDL value was recorded by group (I/C) (40.8±17.4) in comparison to the control group (A) (16.8±2.21) and also in comparison to groups (I/D and I/B) (22.6±6.8 and 14.2±3.35, respectively). The triglyceride value was low in (I/C) group (97.8±11) and was high in (I/D) group (151.6±50.4), while the triglyceride value was (131.4±29.4) in the control group. All cholesterol values were very high in all infected groups (I/C, I/d, and I/B) (167±82.9, 126±47.4 and 85±14.2) when compared to the control group (62.5±5.1).

Different factors, including organ rupture, hemolysis, muscular injury, nutritional state, physical activity, therapy, and infection with pathogenic bacteria, affect liver functions, so the disturbance in levels of liver enzymes in the blood occurs [26].

Previous studies showed that long-term use of high doses of isotretinoin causes autoimmune hepatitis with a significant rise in liver function tests and increases plasma triglycerides in patients [26]. Isotretinoin use results in liver changes that include higher liver enzymes and lipid alterations such as higher triglyceride, total cholesterol, and low-density lipoprotein cholesterol concentrations and decreased high-density lipoprotein cholesterol concentrations [26]. Isotretinoin toxicity in experimental animals affects enzymatic anti-oxidants, glutathione levels, and the activity of catalase in liver tissues, leading to greater abnormalities in the function of the liver [27]. Low-dose isotretinoin has few side effects when used as a treatment for patients, but high doses lead to biochemical abnormalities such as hyperlipidemia and impaired liver function tests [27].

Table 3. Comparison of Liver function and lipids between control and three infected groups of rabbits (I/D, I/B, and I/c) treated with (xeractan, xeractan with beetroot, and Chinese ginger)

Groups	AST(U/L)	ALT(U/L)	LDL (mg/ dL)	HDL (mg/ dL)	TG (mg/ dL)	Chol (mg/ dL)
A	55.7±19.1	70.7±18.5	20.25±1.5	16.8±2.21	131.4±29.4	62.5±5.1
I/D	71.7 ±28.9	57.2 ±16.09	74±34.1*	22.6±6.8	151.6±50.4	126±47.4
I/B	77.8±39.3	78.9±31.5	48±11.6*	14.2±3.35	111.6±34.7	85±14.2*
I/ C	169.4±45.2*	160.5±32.03*	117±59.9*	40.8±17.4*	97.8±11	167±82.9*

Chol, cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

In our study, the value of AST, ALT, and HDL in (the I/D) group is nearly close to the control group, which means the treatment with isotretinoin has no side effect on this parameter but has high side effects on LDL, TG, and Chol values because the values higher than that recorded in the control group. The previous studies indicate that the small dose of isotretinoin did not affect the level of AST and ALT enzymes; the level of enzymes increased gradually by increasing the dose [28].

A previous study that investigated the effect of high-dose isotretinoin treatment in 300 acne patients found mild, asymptomatic transaminase increases in 50% of cases [28]. Although moderate hepatic enzyme increases associated with isotretinoin therapy have been well recorded, there is a similar return to baseline following drug cessation [29].

Of the 130 patients who received isotretinoin, only 70 were treated for 3 months or more. There was a statistically significant increase in the levels of ALT (18.25 ± 8.32 versus 23.35 ± 20.04 U/L), AST (20.45 ± 6.27 versus 24.39 ± 11.93 U/L), and triglycerides (87.02 ± 48.26 versus 105.33 ± 48.77 mg/dL). In other studies, those who took isotretinoin for nine weeks or longer showed increases in the LDL-cholesterol fraction, a decrease in the HDL fraction, and an increase in triglycerides [30].

Red beetroot (*Beta vulgaris*) is an herbal plant that contains the most important phytochemicals, betalains, betaine, and nitrates, which provide benefits beyond normal health maintenance. Extracts of beetroot lower blood sugar and cause the liver to store glucose as glycogen [31]. Experiments on rats demonstrated that an extract of red beetroot decreases the levels of hepatic enzymes such as ALT, AST, and ALP [31].

Beta vulgaris extract contains betaine (trimethylglycine), which has a role in lipid metabolism, involving a decrease in the accumulation of hepatic triglycerides. It also affects the concentrations of cholesterol in plasma and various animal body tissues [32]. GOT and ALP are liver enzymes that were significantly decreased by dietary betaine supplementation [32, 33]. Marai *et al*. found that experimental rabbits that took an extract of beetroot during the hot summer had higher serum transaminase activity (GOT and GPT). In geese, the level of lactate dehydrogenase and alanine transaminase was significantly increased when betaine was used as a treatment. As a result, it is possible to assume that betaine supplementation helps to keep serum transaminase enzymes at normal levels under different conditions [34, 35].

The values of AST, ALT, LDL, and Chol in the infected group, which was treated with xeractan with beetroot, were higher than that recorded in the control group, while the values of HDL and TG were low in comparison to values in the control group, all of these values which recorded in (I/B) and (A) were nearly closed to normal values which demonstrated by different references, that means the beetroot extract improvement the bad side effect of xeractan.

All recorded values of (AST, ALT, LDL, HDL, and Chol) in the (I/C) group achieved high values in comparison to values in the Control group, except the TG value was very low in comparison to the control group. All of these values in the (I/C) group were very high in comparison to normal values according to previous references, which indicates that Chinese ginger does not affect liver functions. These results do not agree with the results of previous studies, which recorded that the extract of Chinese ginger plays an important role in lowering serum cholesterol triglycerides and increasing the amount of high-density lipoproteins [36, 37]. Ginger extract has the ability to change lipid metabolism by suppressing cellular cholesterol biosynthesis, boosting bile acid biosynthesis to remove cholesterol from the body, and raising fecal cholesterol excretion [37].

Using ginger capsules by patients with hyperlipidemia for 45 days leads to a lowering of lipid levels in their bodies [38, 39]. Ginger may have a hypolipidemic impact because it stimulates the conversion of cholesterol to bile acids, which is a significant route by which cholesterol is eliminated from the body, as well as because it inhibits cellular cholesterol. These results, however, were in disagreement with those of Rong *et al*. (2009), who stated that giving male and female rats ginger powder treatments up to 2000 mg/kg for 40 days had no effect on their serum levels of triglycerides and total cholesterol. The differences could be related to the varied doses and genders employed in the two tests [40]. Other studies found that the use of ginger extract enhanced liver functions as it lowered liver enzymatic activity; taking ginger extract (200 mg/kg) orally from days 15 to 20 significantly decreased levels of AST, ALT, ALP, and tissue lipid peroxide [41, 42].

The results of **Table 4** showed the values of urea and creatinine as the indicators for kidney function in all treated groups of rabbits (I/B, I/C, and I/D) in comparison to the control group (A). There was a highly significant difference in the values of urea and creatinine in group (I/C) only ($P< 0.05$) in comparison to the control group (A). The value of urea was high in group (I/C) (62.8±5.9) when compared to the value in group (A) (48.9±12.2) while the values of urea in groups (I/B &I/ D) were low (47.9±26.6 and 44.9±17.7, respectively) when compared to the control group, the values of creatinine in groups (I/C & I/D) were high (1.34±0.089 &1.22±0.18) in comparison to the value in the control group (1.15±0.11) but the value of creatinine of (I/B) group was low (0.99±0.151) when compared with the value in the control group (1.15±0.11).

Table 4. Comparison of kidney function between control and three infected groups of rabbits (I/D, I/B, and I/c) treated with (xeractan, xeractan with beetroot, and Chinese ginger)

Groups	Urea	Creatinine
A	48.9±12.2	1.15±0.11
I/D	44.9±17.7	1.22±0.18
I/B	47.9±26.6	0.99±0.151
I/C	62.8±5.9*	1.34±0.089*

The concentration of urea in the blood of Staphylococcus aureus-infected rabbits reduced significantly in comparison with the control group from 72 h to 14 days following infection, while creatinine increased from 81.8 ± 5.7 μM / L to 92.4 ± 4.7 μM / L on the 15th day following infection. Many different previous studies showed the elevation of urea and creatinine, which may be caused by impaired liver and kidney function [40]. In rabbits treated with an albendazole compound for 60 days after being infected with Encephalitozoon cuniculi, Concova *et al*.'s study [43] discovered a significant reduction in urea concentrations. Creatinine and urea levels increased in rabbits that had been experimentally infected with coccidia of the species Staria Cervi, as reported by Kumar and Josh, who examined a longer time period (3 months following infection). Also, a credible urea increase of 19.63 ± 2.79 to 47.86 ± 5.55 mM/L during the 30th - 70th day of infection was detected in another study. The difference in urea level in different studies may be that urea is the end product of the ornithine cycle's detoxification of toxic ammonia, and ammonia is created when amino acids are deaminated during the catabolism of proteins and when the purine bases of nucleic acids are deaminated. Although the kidneys are responsible for excreting the urea into the urine, it is synthesized in the liver [43]. Hemolysin exotoxins and pyrogenic toxin superantigens (PTSAgs), which are absorbed and carried by the blood into the liver and rens, are the two forms of exotoxins produced by *S. aureus* [44]. The harmful effects of *S. aureus* affected not only the skin but also the liver and kidney, as PTSAgs may disrupt liver endotoxin clearance capabilities via direct cytotoxic impacts on the cells of the liver, which results in harmed hepatocytes and impaired the function of the above-mentioned organs [45]. Small changes in levels of urea are hard to interpret, as urea concentrations in rabbits are affected by physiological factors such as the circadian rhythm (peaking in the late afternoon and early evening), the quality and quantity of proteins in the diet, the function of the liver, intestinal absorption, the activity of urease in the caecal flora, nutritional status, and the state of hydration [46].

In our study, the value of creatinine in the infected group, which was treated with isotretinoin (1.22±0.18) higher than the value that was recorded in the control group (1.15±0.11); this result did not agree with the result of Parlak *et al*. [47] who study the effect of isotretinoin on creatinine of blood sample for rat, Creatinine level was not significantly changed in vehicle-treated nephritic rats (1.5 ± 0.15 mL/min/kg BW) in comparison to non-nephritic control rats (1.4±0.3 mL/min/kg BW) in the presence of either low dose or high dose isotretinoin treatment.

In a previous study by Parlak *et al*. [47], which aimed to study the effect of taking isotretinoin by patients with acne vulgaris, the results revealed that there was an increase in the level of creatinine on the third month of taking treatment while the value of urea was not changed under the action of isotretinoin.

The values of urea and creatinine of group (I/B) are nearly close to values recorded in the control group(A), which shows that the treatment of infected groups by a mixture of isotretinoin and extract of beetroot may improve the kidney functions of infected rabbits with *Staphylococcus aureus*.

Parlak *et al*. the research conducted [47] aimed to study the effect of dexamethasone, which induces damage to the kidneys of rats, and also the protective effect of beetroot extract against kidney damage by this substance. The results showed an increase in the level of urea and creatinine (48 ± 5.2 &0.68± 0.02, respectively) in a group of rats injected with dexamethasone when compared to the control group, whereas the urea and creatinine values were (39.1±4 & 0.566± 0.05, respectively), the values of urea and creatinine in a group of rat which treated by extract of beetroot as a protective agent were (36.0± 3.5& 0.55 ± 0.08, respectively) these results nearly close to control group and that indicate the role of beetroot extract as protective agents.

The values of urea and creatinine of the (I/C) group were very high in comparison to values recorded in the control group (A), which showed that the treatment of infected groups by extract of Chinese ginger may be made disturbance of kidney functions of infected rabbits with *Staphylococcus aureus*. Ajith *et al*. [48] studied the effect of the extract of Chinese ginger on the level of urea and creatinine in the blood serum of rats; the results revealed that the level of urea decreased while the level of creatinine increased under the effect of ginger extract when compared to the level of both parameters in the control group and the difference were not statistically significant. Our results did not agree with the results of previous studies, which noted significantly increased serum creatinine concentration and decreased serum urea in blood serum in cases treated with extracts of Chinese ginger because ginger contains flavonoids and polyphenols that influence the removal of certain waste products from plasma. The chemical compound creatinine, which is produced by the degradation of creatine phosphate during the metabolism of muscle proteins, is filtered at the glomerulus and then removed from the plasma via the kidney. This means that although urea is filtered and partially reabsorbed in the nephron, creatinine is only filtered and not reabsorbed; ginger may have a minimal effect on its excretion. Additionally, extracellular constriction caused by the relationship between urea and the reabsorption of water could result in increased concentrations of chemicals like creatinine in plasma [48], which may explain why ginger-treated rabbits had a much higher level of creatinine.

Conclusion

The rabbits are a good example of laboratory animals for studying the harmful effects of infection with specific *Staphylococcus aureus*. In our study, the clinical signs varied according to the health status of the rabbits. The extract of *Beta vulgaris L*. plays an important role in the treatment of side effects of using xeractan only and may improve the blood values liver and kidney functions of infected rabbits. Also, an extract of *Kaempferia pandurate* may treat the harmful effects of infection with *Staphylococcus aureus* and may improve the blood values liver and kidney functions of infected rabbits.

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