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Evaluating the *Symphytum officinale* effect against *staphylococcus aureus* that induced liver pathochanges (histological study)

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Abstract

The current work was aimed to estimate the potential role of *Symphytum officinale* to improve the liver tissue against pathochanges induced by *staphylococcus aureus*. The samples (wound inflammation) were collected from Kirkuk Hospital in Kirkuk city for the period from May to July 2022 from patients and 24 male rats were used and distributed into four groups. The results showed that microscopic and biochemical parameters were used to diagnose bacterial isolates of the genus. The liver enzymes activities in the infected group were significantly higher ($P< 0.05$) than in the control group. In the treated group, liver enzymes were back to normal after utilizing *S. officinale* extract. About histological examination, the cross-sections taken from the control group revealed normal liver tissue, including normal central vein, normal hepatocytes, and normal sinusoids. After utilizing S. officinale extract, livers from the treated group showed semi-normal liver structure, while the infected group displayed minor alterations. It was found that *S. officinalis* extract has an effective antibacterial activity against Gram-positive bacteria.

Keywords: Symphytum officinale, staphylococcus aureus, liver tissue.

Introduction:

Staphylococcus aureus is a gram-positive commensal bacterium that colonizes different body regions in 30% of healthy individuals (1,2). It has a major role in the spread of diseases in hospitals and the general public, which can range from minor to fatal (1, 3, 4). The world is home to several types of Staphylococcus bacteria. Humans who are long-term carriers of *S. aureus* exhibit colonization in their urogenital, gastrointestinal, and upper respiratory tracts in about 20% to 30% of cases (5-7). Animals used as food sources serve as reservoirs (8). Animal skin, including the skin of the teat and teat canal, is colonized by *S. aureus* (9). *S aureus* expresses a wide range of putative virulence factors (10). proteins on surfaces that promote host tissue colonization (11). Examples of substances that most likely inhibit phagocytosis are immunoglobulin binding protein A and capsules (12). poisonous compounds that damage host tissues and cause signs of illness. In general, coagulase-negative staphylococci exhibit fewer virulence factors and are less harmful. It is easy for *S. epidermidis* to colonize implanted devices (13). For more than 2000 years, *Symphytum officinale* (family Boraginaceae) roots and leaves have been used internally and externally

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to treat various diseases (14). Comfrey's pharmacological and therapeutic potential, including neurology, is determined by a variety of chemical components (15,16). The use of plants and their parts to cure illnesses is widespread in many nations, and the World Health Organization (2014) highlighted the significance of traditional medicine, stressing its various forms and particular varieties (17,18). Because of its therapeutic and restorative qualities, comfrey is widely acknowledged as being beneficial for use in pharmaceutical formulations. By assessing the zone widths of growth inhibition, the described research demonstrates the antibacterial properties. The matching extracts from the various plant sections were obtained using different solvents. Overall, the findings showed that comfrey extracts exhibit antifungal activity against *Bipolaris oryzae* and possible antibacterial effects on some examined bacterial species, including *S. aureus* (19,20). The endophytic strains from *S. officinale* leaves may be biologically controlled to inhibit *S. sclerotiorum*, according to Rocha et al. (21). Furthermore, comfrey leaf extracts reduced the vulnerability to infection from *Erysiphe graminis* conidia and *Puccinia graminis* uredospore in wheat steam, according to Karavaev et al. (22). Many substances, such as phenolic compounds and other bioactive molecules found throughout the plant, may have affected the effect of the extracts under study (23, 24). Therefore, the current research sought to understand how *S. officinale* might protect liver tissue from pathological changes brought on by *Staphylococcus aureus*.

Material & Methods:

Clinical specimen collection:

From May to July 2022, clinical samples (wound inflammation) were taken from patients admitted to Kirkuk Hospital in Kirkuk City who had been seen by a specialist physician who had referred them to the lab.

Bacterial Identification:

The following criteria were used to identify bacteria: morphological analysis, media properties, microscopic examination, and biochemical tests.

Preparation of extracts:

25 grams of the thoroughly cleaned and chopped *S. officinale* root and 100 milliliters of distilled water were placed in an electric grinder one at a time. Following centrifugation, the result was considered to be 100%. Following that, study preparations were carefully considered.

Experimental animals:

The 24 adult male rats that were acquired from a pharmaceutical research institution in Baghdad and an Iraqi care facility were divided into four groups of six rats each:

- Control group: received normal saline (orally).
- Infected group: injected with 10^6 CFU of *S. aureus* (intraperitoneal) (25).
- Extract group: received *S. officinale* 150mg/kg BW (orally).
- Treated group: treated with *S. officinale* 150 mg/kg after being injected with 10^6 CFU of *S. aureus.*

Prepare of blood:

Blood was drawn from rats by heart puncture and placed in test tubes with EDTA. The AST, ALT, and ALP activities of the serum were calculated.

Histological study:

After infection, mice were put under anesthesia and sacrificed. Samples of liver tissue were collected and preserved in 10% neutral buffered formalin for histological examination. Following standard histokinette preparation, the samples were sliced with a microtome into slices that were 5 mm thick, stained with hemotoxylin and eosin stain, and viewed under a light microscope (26).

Statistical analysis:

For analysis of statistics the results were expressed using means and SE. Analysis of variance

(ANOVA) was utilized to statistically examine the data and identify any differences between the groups before and during the therapy. P <0.05 was deemed statistically significant in the data analysis conducted using SPSS (SPSS 2003, SPSS Inc.) (27).

Results & Discussion:

Isolation and identification:

The size and morphology of *S. aureus* isolates cultivated on Mannitol Salt Agar are shown in Figure (1). On the other hand, bacterial isolates of the genus were identified using microscopic characteristics such as the interaction of the Gram stain. Colony traits like color, texture, and pigment production were also utilized as criteria to distinguish isolates and genera.

Liver enzymes:

The results of the investigation showed that the liver enzymes of the groups differed significantly (P<0.05). Table 1 shows that the infected group's AST levels were substantially higher (P<0.05) than those of the control group. Compared to the control group, the infected group's ALT and ALP levels were significantly higher (P<0.05). Following the use of *S. officinale* extract, the treated group's levels of AST, ALP, and ALT showed non-significant variations (P<0.05) in comparison to the control group.

El-Gendy et al., (28) reported that serum levels of ALT and AST significantly increased, which was indicative of a hepatotoxic effect induced by MRSA extracts (p<0.01). Similarly to this, our research showed that treating male mice with MRSA isolates increased their levels of ALT and AST significantly in comparison to the control and other treated groups. These findings suggested that MRSA isolates have the potential to be genetically toxic (28,29). Regarding the *S. officinalis* treatment. Treatment with *S. officinalis* in the current study prevented the increase in liver enzymes that *S. aureus* caused in rats. Previous research has demonstrated that *S. officinalis* possesses potent antioxidant qualities. Horvathova et al. discovered that rats' drinking water contained *S. cerevisiae* germs. Rat hepatocytes are more resistant to oxidative stress when officinalis extract is used. It protects hepatocytes from hydrogen peroxide and dimethoxy naphthoquinone-induced DNA damage by boosting glutathione peroxidase activity (30,31). According to Zupko et al. (32), the antioxidant components in the Salvia extract—salvianolic acid, rosmarinic acid, and phenolic glycosides—were responsible for this action.

Liver tissues

The control group's cross-sections showed normal liver tissue, including normal sinusoids, hepatocytes, and central veins (Figure 2). Figure 3 depicts the infected group's thickening wall, central venous congestion, significant lymphocyte infiltration, and hepatocyte degradation. The liver tissue architecture was normal in the cross sections of this group that were given *S. officinale* extract (figure 4). Following the use of *S. officinale* extract, liver slices from the treated group showed semi-normal liver tissue architecture (figure 5).

In the current study, mononuclear cellular infiltration was found during the histological examination of liver tissue infected with *S. aureus*, particularly in proximity to dark regions that are believed to be *S. aureus* colonies. The mild widespread inflammation in the liver is most likely caused by bacterial endotoxins, which change the shape of cells (33). In this study, the affected liver sections had swollen, necrotic, vacuolated, and degraded hepatocytes. Additionally observed were pyknosis of the blood sinusoids and nuclei, as well as congestion of the central and portal veins. According to a study on the pathophysiology of experimental *S. aureus* infection in rabbits, the majority of these histological findings were in line with the findings that the infected animals' livers had bile sinusoid obstruction with bile pigment, hydropic degeneration and necrosis of hepatocytes, and liver congestion (34). It's interesting to note that most of the negative histological and ultrastructural effects

of the *S. aureus* infection on the liver were considerably lessened by oral administration of the S. officinalis aqueous extract, and the majority of the hepatocytes' cytoplasm and nuclei normalized. In a similar vein, Amin and Hamza (35) found that mice pretreated with S. officinale extract did not show signs of hepatic necrosis or inflammatory cell infiltration in the liver following azathioprine treatment.

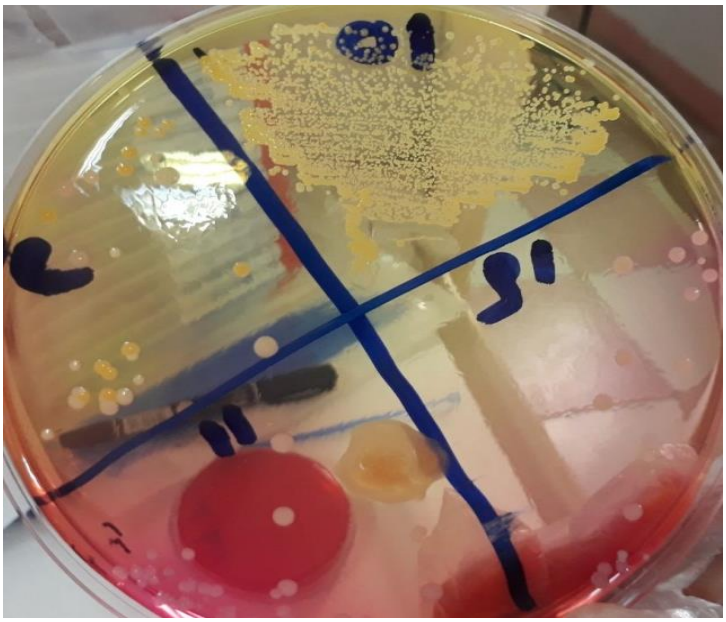

Figure (1): S. aureus on Mannitol Salt Agar

Table (1): Levels of liver enzymes in the studied group

Variables / Groups	AST U/L	ALT U/L	ALP U/L
Control group	17.44±7.59	21.53±5.33	52.17±9.52
Infected group	78.93±9.84*	94.05±11.27*	186.31±17.06*
Extract group	19.01±3.41	21.42±6.15	49.38±3.39
Treated group	28.47±6.72	30.58±9.48	59.42±8.27
P value	0.0001	0.001	0.0001

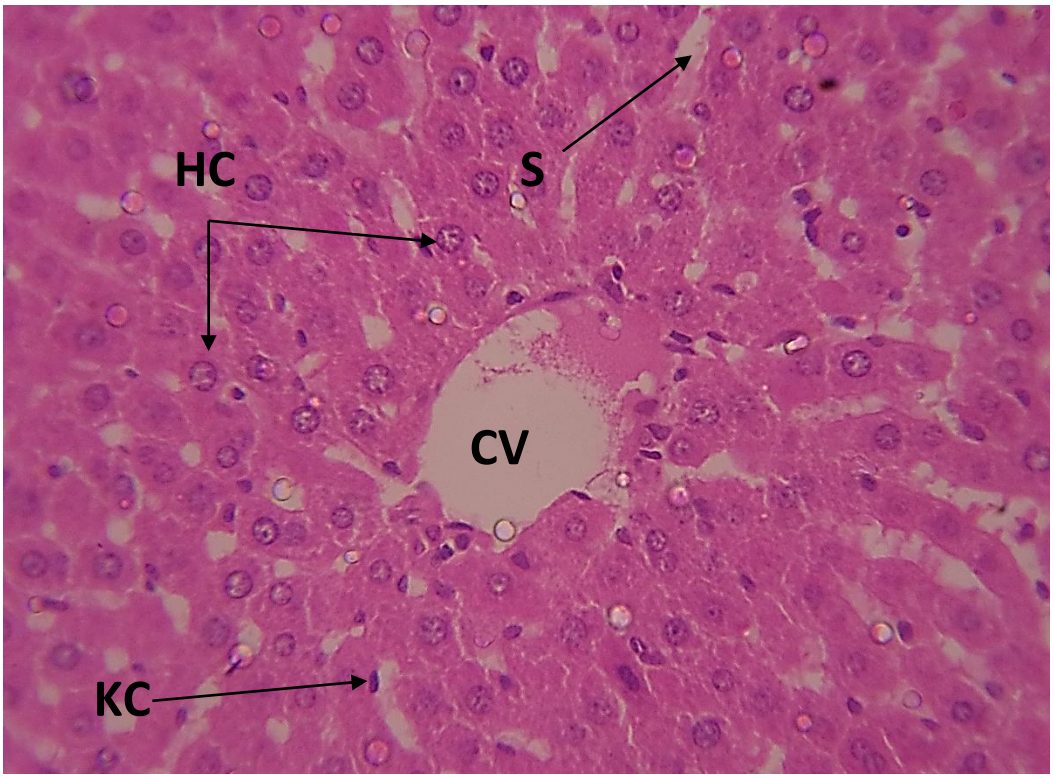

Figure (2): control group A showed the normal structure of the central vein (CV), hepatocytes (HC), sinusoids (S), and kupffer cells (KC) H&E X400.

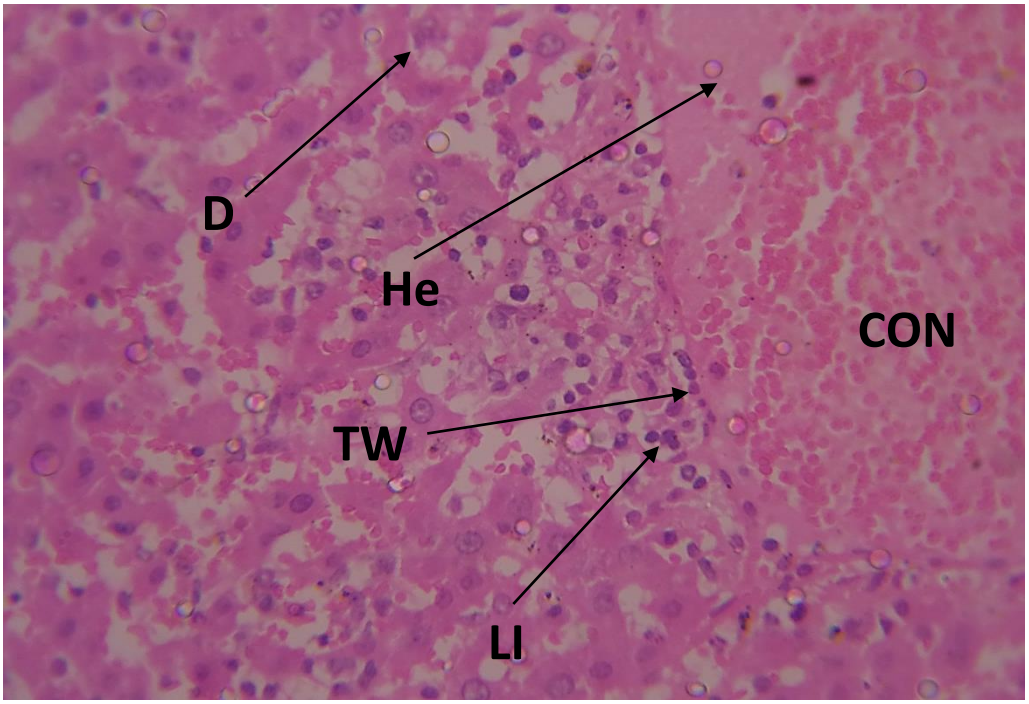

Figure (3): infected group showed thickening wall (TW) of central vein (CV), with congestion (CON), lymphocytes infiltration (LI), and Degeneration (D) of hepatocytes and blood hemolysis (He) H&E X400.

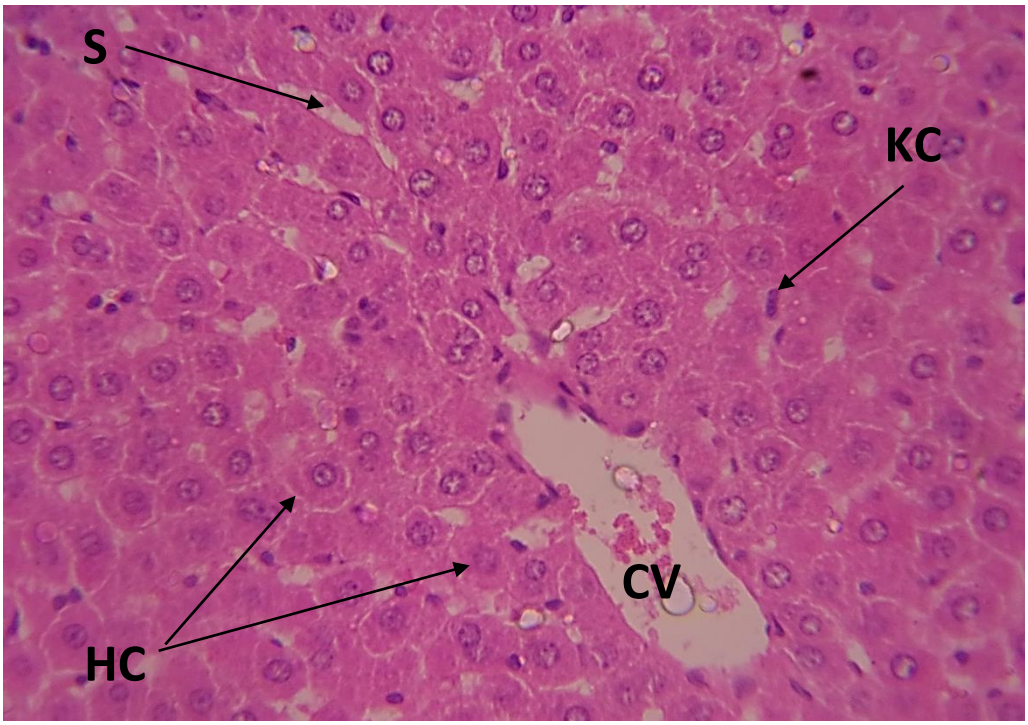

Figure (4): extract group showed central vein (CV), hepatocytes (HC), sinusoids (S), and kupffer cells (KC) H&E X400.

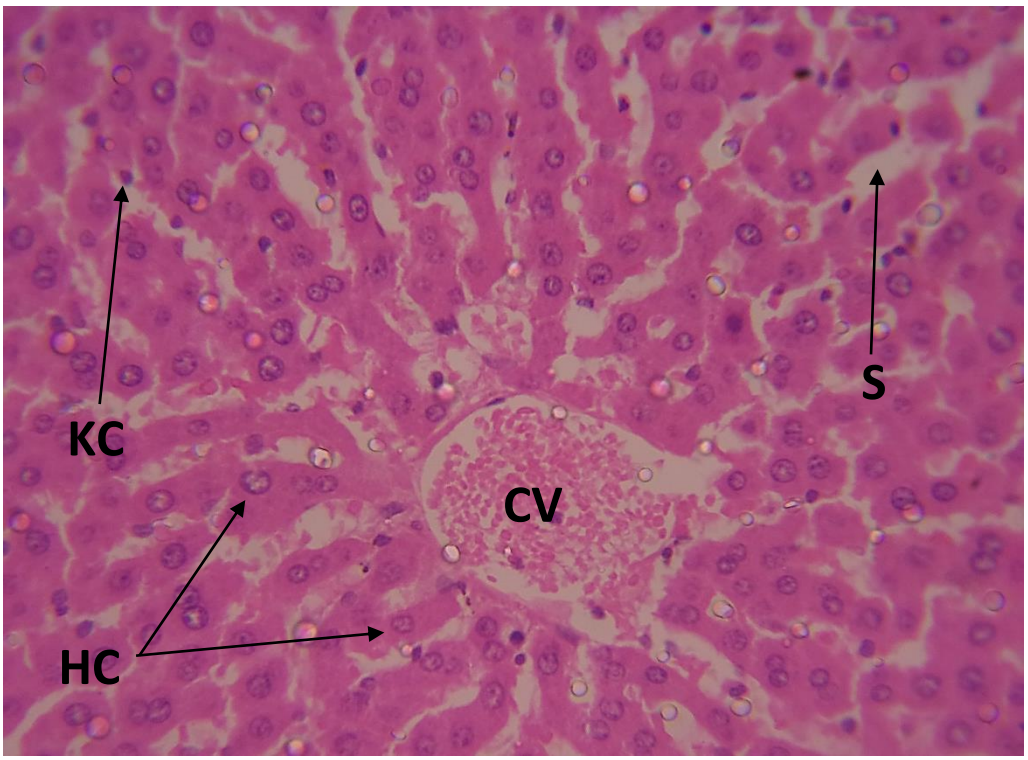

Figure (5): Treated group showed central vein (CV), hepatocytes (HC), sinusoids (S), and kupffer cells (KC) H&E X400.

Conclusions:

Based on the present investigation, it was found that *S. officinalis* extract has an effective antibacterial activity against Gram-positive bacteria and can protect the liver from bacterial toxins and other microorganisms.

Ethical approval:

This study was approved by the institutional ethics committee. The study protocol and consent form were reviewed and approved by the local ethics committee.

Conflict of interest: NIL

Funding: NIL

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