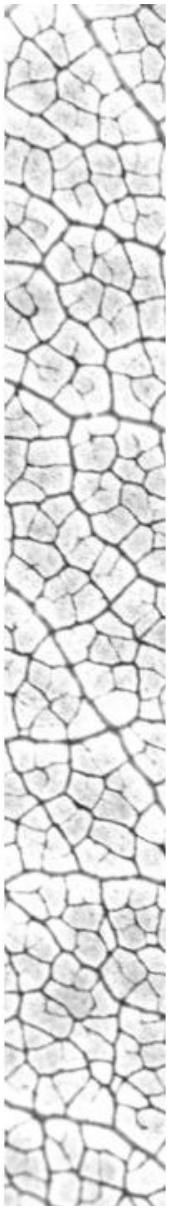

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Histopathological effects of dandelion extracts on Methicillin-Resistant *Staphylococcus aureus* infected in male western Rats

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Abstract
This study investigated the histopathological effects of alcoholic extract of dandelion leaves on infected model animals, male western rats, by methicillin-resistant Staphylococcus aureus (MRSA). The present study has explored the effect the dandelion ethanolic extract using a concentration of 100 mg/l.The experimental rats were infected with a single dose of MRSA; they were then inoculated intranasally by a concentration of MRSA (1.0 × 107 CFU/ml). Negative control was treated with distilled water only. Fifteen rats were randomly divided into three groups equally. The first group was infected with MRSA. The second group was treated with the ethanolic extract of dandelion (0.3 ml per rat) for a month after infected by MRSA. The third group was a negative control, which was administered 0.3 ml of PBS.Thus, the results showed that the ethanolic extract of dandelion can play a crucial role in controlling this pathogen. The histopathological study illustrated the pathological changes in the internal organs of the first and second groups that infected rats with these bacteria within thirty days of treatment. In conclusion, the current study has confirmed the effects of dandelion extract on infected MRSA in Westar rates, illustrated minimal histopathological changes in the lungs, kidneys, heart and liver compared with infected rats.

Keywords: ethanolic extract, dandelion, MRSA, western rats

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INTRODUCTION

For centuries, plants and their extracts have been used in many countries as traditional medicine (Dakheel and Al-Saigh, 2012, Mohammadzadeh et al., 2020, Ullah et al., 2020, and Popova., 2020). Further, after increasing drugs resistance by superbugs, the search for alternative remedies was increased. Although nature provides a different source of medicinal plants that are applied in the modern drug industry for centuries. This medicinal plant has played a vital role in the health of the patients (Seo et al. 2005). World Health Organization reported that around 80% of the population used to be applied the modern drugs instead of using the traditional remedies for their health problems (Ekor, 2014).

Dandelion, a flowering plant, is applied in herbal medicine for decades; it contains many active compounds that exist in its extract. It also showed several pharmacological effects, including healing the swelling, anti-inflammation, antimicrobial and detoxification (Molofsky et al. 2015). However, it is not clear how it inhibits the inflammatory response; therefore, the objective of the current study is to determine the impacts of dandelion on pharmacological aspects that are used to produce several new antibiotics (Ahmad et al. 2000).

Approximately 30% of the nasal cavities of the population has colonised a Gram-positive commensal bacteria, which is named Staphylococcus aureus (Sakr et al. 2018). Further, these bacteria have been found on the skin, nasal cavity and gastrointestinal tract of human (Mulcahy and McLoughlin, 2016). Nonetheless, depending on host conditions, it could cause the infections that related to the individual case or the hospitals and community (Schmidt et al. 2015); therefore, the current study was designed to investigate the histopathological effect of alcoholic extract of dandelion leaves on infected animals by methicillin-resistant Staphylococcus aureus (MRSA).

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MATERIALS AND METHODS

Study area

The present study was conducted at the beginning of June 2019 until the end of August 2019, which is located at the College of Veterinary Medicine/ University of Kufa/ Iraq.

Ethical approval

The protocols that were applied in the current study were approved by the Committee of College of Veterinary Medicine at the University of Kufa, and the approval number was 8/2019.

Preparation of media and nutrient agar

The medium and agar were prepared as described by Seo (2005) for the antimicrobial properties. 8 g of nutrient broth (Merck, UK) was completely dissolved in 1L of distilled and demineralized water. The agar was prepared freshly as dissolved 5 g of Bacto-agar (Difco Laboratories, UK) in 200 ml of distilled water, which is stirred and heated continuously until the solution turns to be clear. Both the media and agar were sterilized by autoclaving at 121°C for 15 - 20 min; afterwards, those were left to settle in and are cool at 50-55 °C.

Bacterial isolation

Nasal carriers, which were isolated from healthy undergraduate students in the Faculties of the Veterinary Medicine/ University of Kufa, were selected for evaluation. Isolate representing MRSA, according to biochemical test, Antibiotic susceptibility test, then confirms diagnosis according to VITEK test.

Culture media

These cultures were prepared freshly and separately according to the product manuals.

1. Tryptic soya agar (TSA), (BBL/Difco)
2. Tryptic soya broth (TSB); (Merck, Germany).

Preparation of bacterial inoculum

According to the protocol that described of Seo et al. (2005), S. aureus stock was prepared to onto tryptic soy agar (TSA), and then it was incubated at 37°C for overnight; further, these colonies were also sub-cultured on Trypticase Soy Broth (TSB) for overnight as well. Afterwards, the McFarland tubes that contained serial dilutions in sterilized saline solution were prepared using 0.9% of NaCl to an appropriate concentration at 1.0 x 107 CFU/ml.

Collecting and preparing the samples

The Dandelion leaves (Taraxacum officinale) were collected from AL- Najaf, Iraq in May 2019. These leaves were transported and dried at room temperature in the laboratory; the samples were authenticated by Dr Sadia (University of Kufa/ College of Veterinary Medicine/ Department of physiology). These samples were then pulverized by mechanical mills; they were weighed and cleaned with filter paper (Whatman No.1) to remove the traces of dust, then dried again in shad at 30-40 ºC for a week. Afterwards, the samples were weighed, ground in a mortar and placed in airtight bottles and stored in a desiccator to be used for extraction (Dakheel, 2018).

Plant extraction procedure

A protocol for the extraction of dried leaves from Taraxacum officinale was established following the method reported earlier by Sarker et al. (2006). 50 g of dried leaves were extracted using a Soxhlet apparatus with a solvent of 15% diethyl ether/ ammonia (90/10) for 4h. The filtrate was rinsed with 4N HCl at room temperature (step A). An organic phase was dried with anhydrous Sodium Sulphate and evaporated to dryness under reduced pressure to give a white precipitate. The precipitate was further purified by dissolving it in chloroform then precipitation by drop-wise addition of methanol. The acidic aqueous phase obtained in Step A was extracted with hexane (A) then ethyl acetate (B), and finally it was treated with ammonia until pH 12 to obtain the alkaloid fraction.

The aqueous/ acid phase was then applied to 30% of ammonia (pH=12), which was accompanied for colour changing to dark green/brown, where it was extracted with dichloromethane three times (1/3, v/v) using a separator funnel. The organic layers were then dried with anhydrous Sodium Sulphate; the solvent was removed under a pressure at 40°C which is given a dark brown-green of alkaloid fraction at approximately 4 g (Koh et al. 2010). This crude extract has been then used for the next experiment.

Experimental animals

Eight-week-old male western rats weighing (22.0 ±2.0 g) were kept in metal cages at room temperature (around 23 °C) for 12h in the dark-light cycle and permitted to freely consume water and food in the animal house. These animals were randomly divided into three groups and treated as follows:

1. The first group (1st; n= 5)

The animals were intranasally inoculated with 10 µl of the isolates containing 1.0 x 107 CFU/m bacterial suspensions, introduced into both nares without touching the tip of the nose

2. The second group (2nd; n= 5)

The animals were intranasally inoculated with 10 µl of isolated samples, containing 1.0 x 107 CFU/m bacterial suspension, introduced into both nares without touching the tip of the nose, then treated with the of Dandelion extract (0.3 ml/rat) for a month.

3. The third group (3rd; n= 5)

This group was served as a negative control, which was intranasally inoculated with 0.3 ml/ of sterile PBS. The post-mortem examination was carried out to all animals and samples were taken for bacterial isolation and others for the histopathological examination.

Specimen preparation for histological slides

The specimen from tissues, including heart, kidneys, liver and lungs, was undergone processes of

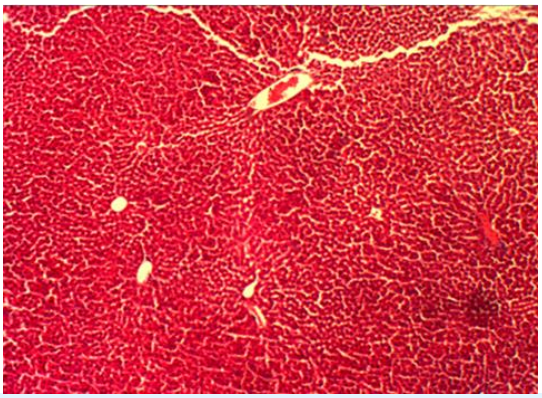

Fig. 1. Histopathological changes in livers of 1st group; which have a vacuolar degeneration hepatocyte necrosis, as well as the inflammatory cells infiltrated in the liver parenchyma. H & E stained organs (100 ×)

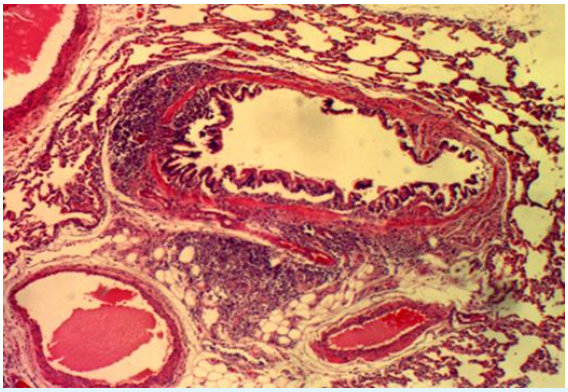

Fig. 2. Histopathological changes in lungs of 1st group; the MRSA infection showed typical destruction of the bronchioles and alveolar architecture, hemorrhage, supportive inflammation that is indicated in the interstitial and perivascular locations. H & E stained organs (100 ×)

dehydration, deparfinaisation and paraffin embedding. As a sequence, the cuts were made with a 3 µm thicker using a microtome (LAB-MR500). Then, these were fixed and stained with Hematoxylin and Eosin stains on slides (Li et al. 2018). These slides were observed using an optical microscope and photographed using an optical camera.

RESULTS

Clinical finding

There are no clear clinical symptoms that noticed on the experimental animals post-infection in all groups through the course of the study.

Bacterial isolation

The results showed that MRSA isolated from the examined animals (7 days post infected rats) that confirmed the diagnosis by VITEK test (Walther et al. 2012).

Pathological study

The infected rats (7-day of post-infection) showed clear gross lesions characterized by splenomegaly and

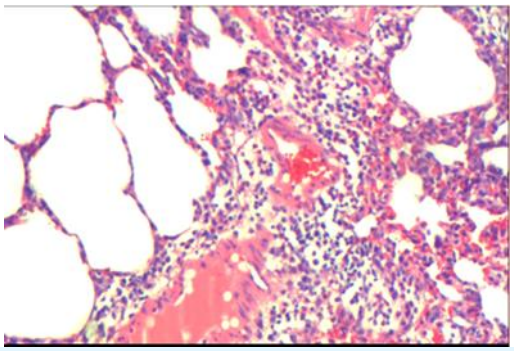

Fig. 3. Histopathological changes in the lungs of the 2nd group, which congested of a blood vessel and hemorrhage with edema, and fibrin aspect (thick arrow). Inflammatory and infiltration cells are indicated in the interstitial and perivascular locations (thin arrow). H & E stained organs (100 ×)

hepatomegaly in both groups which infected either by clinical isolate or nasal carriage isolate in compare with non-infected animals. The liver of these animals in group 1 and 2 revealed yellowish-white spots on the surface. Also, gross examination revealed nodular formation in lung splenomegaly was noticed in these groups. No gross lesions were diagnosed in the control.

Histopathological examination of infected tissues with MRSA

Histopathologic findings of the rat lungs that infected with MRSA at 7-days was performed using Hematoxylin-Eosin stains, considerable haemorrhaging accompanied by the presence of necrosis with an inflammatory infiltrate, (**Fig. 2**). Pathological lesions were consistently higher than the lungs of animals treated with dandelion extracts (**Fig. 3**).

The lungs of several animals, which were infected with MRSA, showed oedema in alveoli, severe necrotizing pneumonia, atelectasis and thick vessel congested necrotic in presenting tissues (**Fig. 4**). Bronchial structure containing inflammatory infiltrate and destruction in their wall, emphysema, with perivascular inflammatory, infiltrate peribronchial inflammatory infiltrates.

The livers in the 1st and 2nd group have shown an acute cellular degeneration with Kupffer cells proliferation and hepatocyte necrosis (**Fig. 1**) compared with the control group. Pathological lesions were consistently higher than the liver of animals that were treated with dandelion extracts. The histopathological sections of infected rat livers, which were treated with dandelion extracts, were also illustrated that the necrosis, fibrosis and portal inflammation at 2nd and 4th weeks of the study compared to the positive control, infected rat livers, at the end of the same corresponding weeks of the experiment showed minimal perioral inflammatory of cell infiltration.

The glomeruli displayed tubules with lining epithelium express necrosis (**Fig. 5**). The

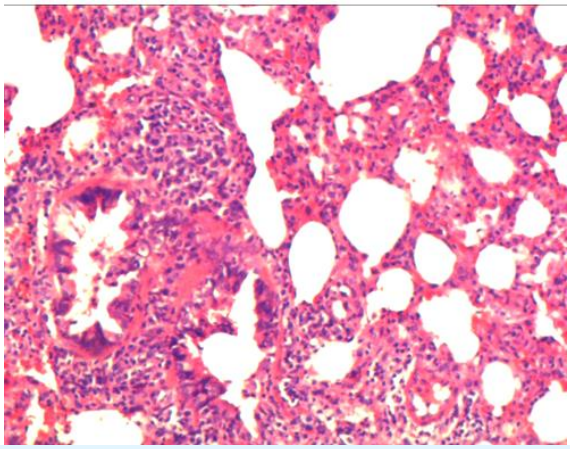

Fig. 4. Histopathological changes in the lungs of 1st group; the peribronchiolar inflammatory infiltrates (white arrow). Mainly PMNS inflammatory cells and thickening in an alveolar wall, proliferation of fibroblast in the bronchiolar wall (thin arrow), atelectasis area, emphysema (black arrow) sloughing of the epithelial cell of the bronchiole. H & E stained organs (100 ×)

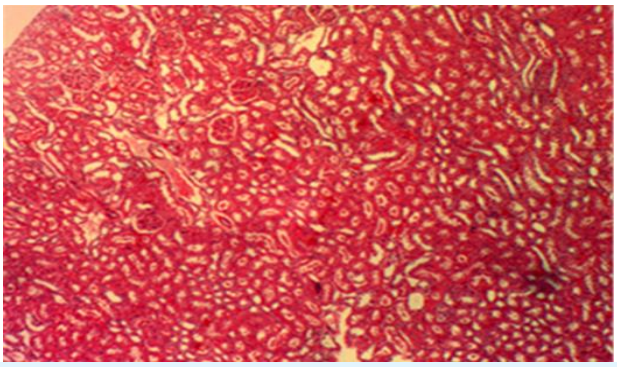

Fig. 5. The kidneys in MRSA infected group showed the neutrophilic inflammatory infiltrate within interstitial tissue, and the presence of a coagulation necrosis area with surrounding tubular mononuclear inflammatory cell infiltrate

histopathological lesions were consistently higher than the kidney of animals treated with dandelion extracts.

No significant differences recorded histopathologically in control groups, which were noted that the cerebral cortex and hippocampus of the brain tissues were in a normal histological structure. In the infected group, the brain and perineural vacuolation were found the most frequent lesions in the cerebral cortex (**Fig. 6**). However, mild congestion of blood vessels and microgliosis findings those were slightly neuronal shrinkage, which was observed in the cerebral cortex of brain tissues in the treated group.

DISCUSSION

MRSA is a pandemic antimicrobial-resistant pathogen; with the increasing rate of Nasal colonization with this pathogen, the risk for development of clinical infection and the chance of high mortality and morbidity rate among carriers is increasing recently (Sakr et al. 2018). Many studies investigated different risk factors leading to the nasal carriage of MRSA among healthy individuals (Schmidt et al. 2015; Mulcahy and McLoughlin 2016). Others mentioned that nasal carriage considered as an important source for spreading infection among susceptible patients and the students are one of the main sources for spreading such pathogen (Mehraj et al. 2016; Laux et al. 2019).

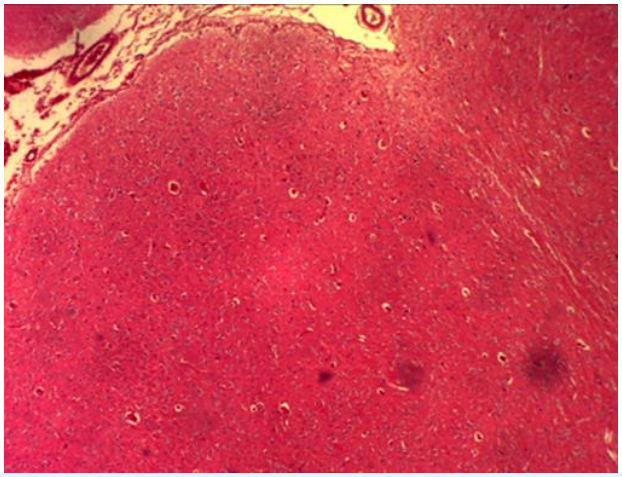

Fig. 6. The cerebral cortex of brain tissues in the 2nd group showed mild congestion of blood vessels, slightly neuronal shrinkage and mild microgliosis

In the current study, it has been investigated the effect of dandelion extract on pathologic MRSA infected western male rats. The active ingredients of plants have provided sources of compounds in the development of new therapeutics (Leal et al. 2000). These compounds naturally occurring plants have the potential to be hepatoprotective and immunoprotective; therefore, those can be considered as the treatment of acute and chronic diseases. Dandelion extract has a variety of pharmacological effects, such as a reduction in swelling and inflammation, and detoxification (Ahmad et al. 2000). It is used in Chinese traditional remedies that contain many active compounds. The in vivo histopathological study of the first and second groups that infected with MRSA showed infiltration of inflammatory cells in some internal organs. The cells of the immune system, such as cytokine/ chemokine, are released to repair the damaged tissue. This can create an environment, which could promote cellular proliferation. Further, these immune cells can also maintain the damaged tissues that have been repaired or replaced with healthy tissue (Shan et al. 2009).

Establishing acute infections can be importantly distinct for the virulence factors from those critical for chronic infections, which are minimally invasive and non-cytotoxic. Both infections may involve in the biofilm formation of human infection; then protected against assault by the immune system of hosts and provided resistance to antibiotics (Simandi et al. 2002). Therefore, the chronic cases could lead to a systemic spread

instead of causing non-productive inflammation, which contributes to induce the morbidity and/or mortality of hosts (Bjarnsholt, 2013).

Studies illustrated histopathologically that the immune system cells for infiltration and destruction of alveolar architecture in lungs at (2.0 × 108 CFU/ml) of bacterial concentration (Ríos et al. 2000; Koh et al. 2010). Similarly, the inflammatory cases were observed in the current study. Another study reported that colonized organisms in nasal cavity could cause sepsis in hospitalized patients. This confirmed that β-lactam antibiotic-resistant strains caused increasing the infections in hospitals (Laux et al. 2018). Additionally, the results showed that the strains isolated from human infections had a higher inflammatory response in lung, kidney, liver, and heart tissues in the animal model, when compared to strains that isolated from nasal cavity in healthy people. The explanation of these results is likely from increased expression of *S. aureus* regulatory systems. These results confirmed that this model is sufficiently reproducible to study bacterial and host factors involved in MRSA nasal colonization by using small numbers of animals (Walther et al. 2012).

As a consequence, the present study showed that the treated group of dandelion extracts reduced inflammatory infection. Among the most important compounds in dandelion are sesquiterpene lactones that reported to include the anti-inflammatory and anti-cancer effects, as well as the phenylpropanoids, which demonstrated to influence as inflammation-modulating (Koh et al. 2010). On the other hand, the 1st group showed inflammation cells and infiltration of precipitation of amyloid in these organs, because of Amyloidosis, which is when an abnormal protein called amyloid builds up in your tissues and organs (Molofsky et al. 2015). A systemic disorder could characterize by the extracellular deposition of a protein-like material in multiple organs; this disorder might lead to progressive organ dysfunction. Similarly, secondary amyloidosis, which is derived from the inflammatory protein serum amyloid A, occurs with chronic inflammatory disease. This serum protein may lead to isolation without evidence of a systemic disease (Ow and Dunstan, 2014).

These findings could suggest that dandelion extracts have significant impacts on various lymphocyte cells in body tissues; further, the results could indicate that dandelion extracts can modulate the immune reactions by credited with the keys of intermediaries in immune interactions (Liu et al. 2018). Further studies are needed to detect the effect of dandelion extracts on the immune system.

CONCLUSION

This study found that dandelion extract can play a vital role in controlling of MRSA infection, causing a significant effect in 2nd group, treated by dandelion extract, and minimal histopathological changes in lungs, kidneys, heart and liver compared with infected rats with MRSA, which is reflected the relative safety and used for therapeutic purposes.

However, the histopathological study showed pathological changes in the internal organs of the 1st and 2nd groups infected with bacteria compared to the negative control group.

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