Annals of Biology **39** (2) : 225-228, 2023

Impact Score : 0.32 (Scopus)

Induction of Kidney Lesions in Rats by *Streptococcus pyogenes* and Evaluate the Herbal Component Activity of *Symphytum officinale* Extract

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(Received: January 2, 2023; Accepted: February 11, 2023)

ABSTRACT

The current work was aimed at investigating the activity of *Symphytum officinale* extract to treat the kidney lesions induced by *Streptococcus pyogenes*. The throat swabs were collected from children patients in Azadi teaching hospital during March to July 2022. Twenty-four male albino rats were placed into four groups each of six rats; negative group which received orally normal saline (0.5 ml/kg) BW; positive group in which injected (intraperitoneal) 10^5 CFU of *S. pyogenes*; extract group which orally received *S. officinale* 150 mg/kg BW and treated group in which injected (intraperitoneal) by 10^5 CFU of *S. pyogenes* and treated by *S. officinale* 150 mg/kg. There were significant variations between groups in oxidative stress and antioxidant enzymes then the control group. The MDA levels in bacterial infected group were significantly higher, while GSH and catalase levels in bacterial infected group were significantly lower. The treated group's levels of MDA, GSH and catalase revealed non-significant changes after utilizing *S. officinale* extract. In the histological study, the microscopic examination of the kidney's cross section revealed various lesions following infection, including damage to the glomerulus, tubule cell deterioration, lymphocyte infiltration and thickening of blood vessel walls. After treatment with extract, cross sections showed semi-normal structure of glomerulus and semi-normal form and diameter of convoluted urinary tubules. In conclusion, *S. officinale* extract can be efficient for decreasing population of *S. pyogenes* and treating infections for severe anti-oxidative effects.

Key words: *Streptococcus pyogenes*, *Symphytum officinale*, kidney, oxidative stress

INTRODUCTION

Streptococcus pyogenes, a Gram-positive, causes non-invasive, invasive infections, in addition to non-suppurative sequelae. The illnesses addressed include glomerulonephritis, streptococcal toxic shock syndrome, cellulitis, scarlet fever, acute rheumatic fever and type II necrotizing fasciitis (Avire *et al.*, 2021). There are currently 18.1 million people who have a severe GAS disease, and there are 1.78 million new cases and 500,000 fatalities every year. Distinct from other groups by using pyrrolidonyl arylamidase and the Voges-Proskauer reaction, some streptococci are typically made simple (Vlad *et al.*, 2017). Along with the Lancefield antigen, the M and T surface antigens play a critical role in the categorization of *S. pyogenes*. The fibrillar M protein, which is located on the outside of cell walls, is produced by the emm gene. Functionally, the M protein inhibits complement deposition, interacts with a variety of host proteins, is pro-inflammatory, and promotes mucosal adhesion in the absence of opsonizing antibodies (Valderrama *et al.*, 2017). The most widely used species is *S. officinale*, also known as herb comfrey (Avila *et al.*, 2020). For more than 2000 years, comfrey has been recommended as a botanical preparation for both external and internal use (Avila *et al.*, 2020). In other parts of the world such as in Navarra ethnographic research has revealed the external use of *S. officinale* also for osteoarticular disturbances while in Mexico the tea for hepatic disruptions and as an internal therapy for rheumatism, in Brazil gastritis and abscesses while skin conditions in the United states of America, and roots for osteoarticular pain in Lithuania or as tonic in Jamaica (Salehi *et al.*, 2019). Medicinal plants have occupied an important status in the search for efficient methods to treat different diseases in addition to discover novel substances because of the currently high prevalence rates of germs gaining acquired resistance to the antibiotics (Hasan and Abass, 2019; Hasan *et al.*, 2020; Hasan and

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Mohammed Bakr, 2022). The use of medicinal plants in the treatment of disease is widespread in many nations, and WHO (2014) reported on the significance of medicinal plants, highlighting its various manifestations and distinctive types. On the other hand, phenolic compounds, essential oils, or antimicrobial peptides are some of the most well-known naturally occurring antimicrobial compounds derived from plants (Lee and Paik, 2016). However, alkaloids, which are organic compounds found in medicinal plants naturally, have been shown to have broad-spectrum antibacterial effects (Fialova *et al.*, 2017). Therefore, the current research sought to examine the ability of *S. officinale* extract to treat kidney lesions brought on by *S. pyogenes.*

MATERIALS AND METHODS

The children who were admitted to the Child Hospital at Azadi Teaching Hospital between March to July 2022 provided the throat swabs. On blood agar plates and azide agar plates, the swabs from the tonsils and post pharyngeal were inoculated. The plates were diagnosed after 18 -24 h incubation period at 37°C with 5-10% CO_2.

Twenty-five grams of the well-cut and cleaned *S. officinale* root were added to an electric grinder along with 100 ml of distilled water. These were then centrifuged, and the outcome was regarded as 100%. Careful consideration was given to study preparations.

The current study used 24 mature male rats of average body weight of 175 to 200 g, and age of 12 to 16 weeks. The clinically healthy rats were housed in metal cages and glass bottles under sanitary conditions. Food and water were freely available during the entire trial. Four groups of six rats each were formed from the 24 male albino rats:

Negative group received normal saline (0.5 ml/kg) BW.

Positive group injected (intraperitoneal) by 10^5 CFU of *S. pyogenes.*

Extract group received orally *S. officinale* 150 mg/kg BW.

Treated group injected (intraperitoneal) by 10^5 CFU of *S. pyogenes* and treated by *S. officinale* 150 mg/kg.

Rats were cardiac punched to extract blood, which was then put into test containers containing EDTA. The serum's concentrations of MDA, GSH and catalase were calculated. Each rat's fresh heart was quickly dissected, fixed in 10% formalin, and dehydrated with increasing concentrations of ethanol. Tissue samples were dehydrated before being cleaned in two xylene variations, impregnated in two liquid paraffin wax variations, embedded and blocked out. Five µm thick tissue slices were stained with hematoxylin-eosin (Saleh, 2020). SPSS (SPSS 2003, SPSS Inc.) was used to analyze one-way analysis of variance (ANOVA) and find differences between the groups before and after the treatment at P=0.05 for statistically significance. Means and SE were used to express the results.

RESULTS AND DISCUSSION

The size and form of *S. pyogenes* (Fig. 1) displayed *S. pyogenes* isolates on blood and nutrient agars. Whereas microscopic traits like the interaction of the gram stain were used to diagnose bacterial isolates of the genus. Additionally, parameters used to identify isolates and genera included colony characteristics like colour, texture, metallic luster and pigment production.

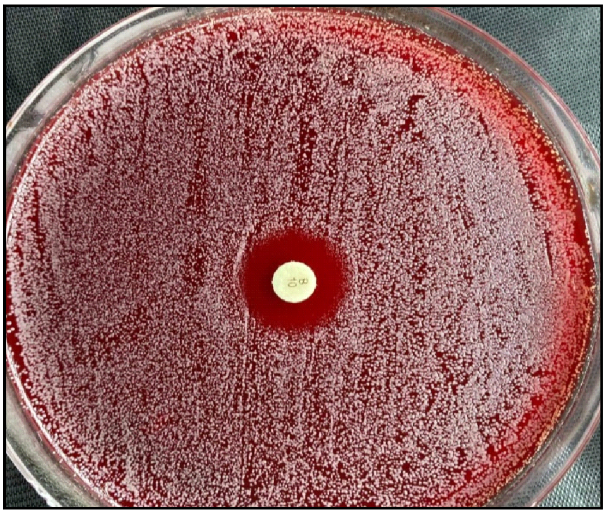

Fig. 1. Colonies of *S. pyogenes.*

The oxidative stress and antioxidant enzymes were significantly different between groups. The MDA levels in the bacterially infected group were significantly higher when compared to the control group (Table 1). While GSH and catalase levels were significantly lower in the bacterially infected group compared to the control group. After using *S. officinale* extract, the treated group's levels of MDA, GSH and catalase showed non-significant changes in comparison to the control group.

The potential of natural substances as antioxidant agents was currently studied using antioxidant characteristics *in vitro* and in biological systems (Gulcin, 2020). Numerous plant-based natural antioxidants were found to function as powerful free radical blockers, reducing agents, or active oxygen scavengers.

Table 1. Levels of MDA, GSH and catalase in the studied group

Groups \ Variables	MDA (nmol/ml)	GSH (nmol/ml)	Catalase (nmol/ml)
Control group	1.48±0.11	0.447±0.037	1.32±0.15
Bacterial group	2.25±0.28*	0.319±0.031*	0.71±0.11*
Extract group	1.29±0.09	0.485±0.045	1.48±0.17
Treated group	1.71±0.24	0.416±0.052	1.21±0.09
P value	0.02	0.001	0.0001

Products made from comfrey were frequently used in conventional medicine for a variety of pharmacological goals. Due to the fact that they support immune function, wound healing, an anti-inflammatory effect and antioxidant effect, oxidation mechanisms and free radicals contributed to these processes (Hussain *et al.*, 2016). Different Symphytum species have been reported to have antioxidant activity in literature.

The cross sections prepared from control group showed the normal structure of kidney tissue. However, the glomerulus appeared normal in the shape and arrangement with normal form and diameter of convoluted urinary tubules (Fig. 2).

The microscopic examination of the kidney's cross section revealed various lesions following infection, including damage to the glomerulus, tubule cell deterioration, lymphocyte infiltration and thickening of blood vessel walls (Fig. 3).

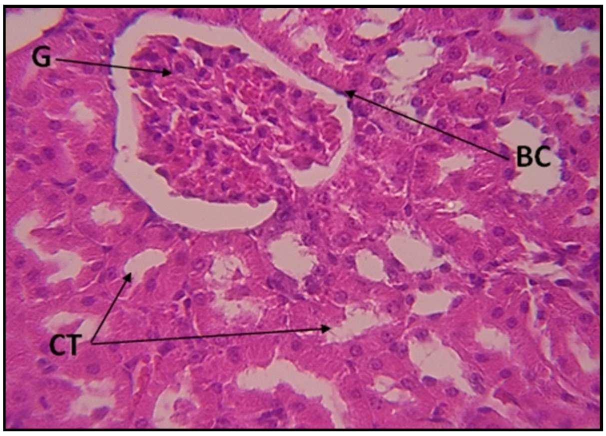

Fig. 2. Convoluted tubules (CT), the bowman capsules (BC) and the glomerulus (G) in the segment of the A group. All had normal structures.

The histological examination of cross sections revealed a normal glomerulus and convoluted urinary tubules with a normal form and diameter (Fig. 4).

The glomerulus's semi-normal structure and the convoluted urinary tubules' semi-normal form and diameter were both evident upon histological examination of cross sections (Fig. 5).

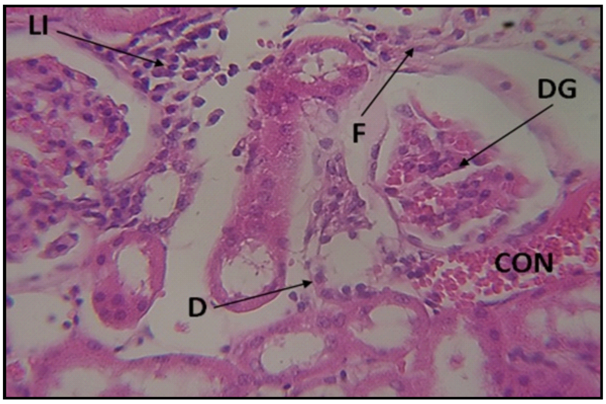

Fig. 3. Section of B group exhibited glomerulus damage (DG), tubule cell degeneration (D), blood vascular congestion (CON), lymphocyte infiltration (LI) and fibrocytes (F) H&E X400.

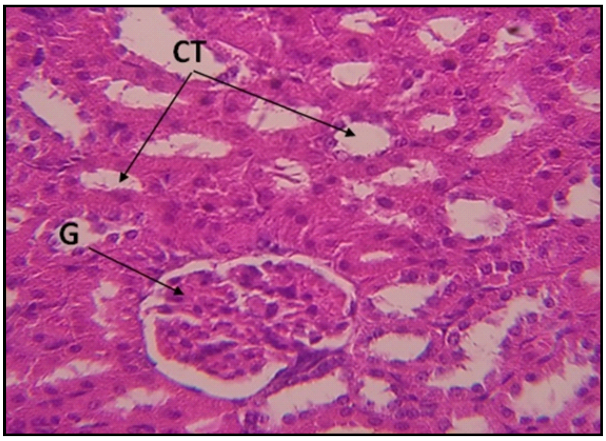

Fig. 4. Section of C group showed the normal structure of glomerulus (G) and convoluted tubules (CT) H&E X400.

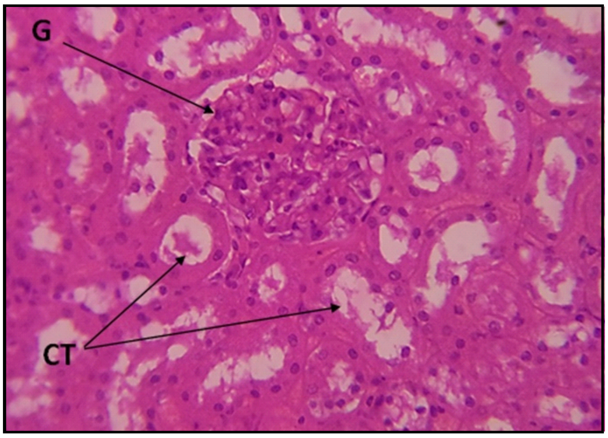

Fig. 5. Section of D group showed the structure of glomerulus (G) and convoluted tubules (CT) H&E X400.

The reason for the presence of different tissue lesions in the kidneys after the infection was brought on by *S. pyogenes* may be explained by the fact that the bacterial infection caused the development of free radicals that brought on all tissue lesions, as evidenced by the results in Table 1. An improvement in kidney tissue was seen after treatment with the extract. The anti-inflammatory and antinociceptive properties of a *S. officinale* root extract with 74.77 g/ml were evaluated by Vostinaru *et al.* (2018). The anti-inflammatory effect of the hydro-glyceroalcoholic extract (500 mg/kg, orally) was evaluated using carrageenan-induced rat paw edema. This extract was reported to be able to reduce edema by 55.6% one hour after inflammation was produced. A glycopeptide derived from the roots of *S. officinal* similarly inhibited the release of prostaglandins and leukotrienes by decreasing phospholipase A2 expression, which in turn had a dose-dependent antichloristic impact on carrageenan-induced rat paw edema (Salehi *et al.*, 2019). Triterpenes, that also inhibit a number of stages of inflammation (including histamine release, cyclooxygenase enzymes and lipoxygenase activity, as well as nitric oxide production), and polyphenols, that also cause a selective COX-2 inhibition, were undoubtedly a large part of *S. officinale's* anti-inflammatory activity (Vostinaru *et al.*, 2018).

CONCLUSION

Symphytum officinale extract can be efficient for decreasing population of *Streptococcus pyogenes* and treating infections by severe anti-oxidative effects with reduction of MDA and stimulation of anti-oxidative enzymes.

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