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Liver Disorders that Induced by Burkholderia Mallei and the Role of Phenolic Compounds of Rheum Ribes in Treatment

Ahmed Hamad Saleh
Histology/Medical Laboratory:
University of Al-Qalam College,

Iraq

Abstract **:The present study was designed to show the potential role of phenolic compounds against the toxicity of Burkholderia mallei. The study used 30 adult male rats that distributed to five groups (each group consist 6 rats); control group that received normal saline, second group rat injected intraperitoneal with B. mallei at dose 10 ^{6} cell/ ml. third group rat injected intraperitoneal with B. mallei at dose 1 0^{8} cell/ ml. fourth group rat injected intraperitoneal with B. mallei at dose 1 0 ^{6} cell/ ml and treated with 250ug/ml of phenolic compounds for four weeks. Keywords—component, formatting, style, styling, insert (**key words**). Fifth group rat injected intraperitoneal with B. mallei at dose 1 0^{8} cell/ ml and treated with 250ug/ml of phenolic compounds for four weeks. Sixth group rat treated with 250ug/ml of phenolic compounds for four weeks. The results show high significant increased (P < 0.05) in levels AST, ALT, ALP and MDA with high significant decreased (P < 0.05) in levels GSH, catalase in second and third groups compared with control group. The results of fourth, fifth and sixth groups show non-significant changes (P < 0.05) in all parameters compare with control group when using Rheum ribes. It was concluded that Rheum ribes has been potential role against the toxicity of B. mallei in adult male rats.**

Keywords: phenolic compounds; oxidative stress; antioxidant; liver functions.

I. INTRODUCTION

Burkholderia mallei is Gram-negative bacteria that cause glanders and melioidosis, respectively (Rogers et al., 2016). B. mallei, the etiologic agent of glanders, is considered an obligate mammalian pathogen (Hatcher et al., 2015). Naturally acquired human cases typically occur among persons with prolonged contact with solipeds, and the major routes of transmission are cutaneous and respiratory. Clinical manifestations are route dependent and include abscesses, fever, pneumonia, dissemination to the liver and spleen causing necrotizing abscesses, and bacteremia (Silva and Steven, 2013) Treatment of glanders is complicated as B. mallei is naturally resistant to multiple antibiotics and resides within the intracellular niche of mammalian host cells (Baker et al., 2017). Rheum species from Polygonaceae family are also important herbal plants to be used in pharmacological research. Especially, Rheum ribes (R. ribes) is most popular one to obtain the raw materials of crude drugs in Asian countries (Tartik et al., 2015). Phenolic compounds are secondary metabolites of plants. These are involved in the defense against ultraviolet radiation and pathogen attacks (Kvasnicka et al., 2008). Several thousands of natural polyphenols have been identified in plants. Phenolic compounds are classified in two groups: flavonoids and non-flavonoids (Pallag Tunde et al., 2016).

II. MATERIALS AND METHODS

Animal model

In this study twenty adult male albino rats, (wt 200-250 gm with age 4-6 month) obtained from Veterinary college/ Kirkuk University, and kept on a standard pellet diet for two weeks to ensure its normal and there aren't any infection Maintaining the Integrity of the Specifications

mallei

Bacterial isolation was isolated from swabs wounds of patients with infected wounds in Al- Jumhuriu hospital / Kirkuk city. Then, the isolated was grown on MaConey agar and blood agar. After that, morphological and biochemical tests were done according to (Hassan et al., 2016).

Plant extract

The dried root of Rheum ribes was purchased at Kirkuk market / Iraq. 10 GM of Rheum ribes powder was put in a pot containing 100 ml of distill water. This was allowed to boil for 15 minuets, after which it was allowed to cool. Then the mixture was filtered by using what mans papers (NO 1). The filtrate was evaporated by rotary evaporator vacuum. The powder extract was placed in dark bottlesand stored in 8° C for further use (Prachi et al., 2017).

Extraction and purification of phenolics

A dried sample of bitter melon l0 g extracted for 30 min. by stirring at 4P o PC with 200 ml of cold aqueous ethanol %65 containing 0.5% Sodium metabisulphite. The homogenate was filtered through four layers of cheesecloth, and the residue was then extracted with two additional

portions (100 ml each) of the same extraction solution as described above. The combined filtrate was centrifuged at 7000 rpm for 15 min. at 4P o PC and residue was discarded. Ethanol was removed from the supernatant by rotary evaporator under vacuum at 35P o PC, and the mass is measured. Pigments were eliminated by two successive extractions with petroleum ether. After addition of 20% ammonium sulphate and 2% metaphosphoric acid to the aqueous phase, the compounds were extracted three times with ethyl acetate. The extracts were combined, evaporated and then dried under vacuum at 35P o PC. The residue was redissolved in methanol (1:1) for analysis (Zhu et al., 2008).

Determination of phenolic compounds

The phenolic compounds of the bitter melon were determined using High Performance Liquid Chromatography (HPLC) (Cai et al., 2003). The absorbance was monitored at 254 nm.C-18 Chromatographic column was used. The mobile phase consisted of 100 % methanol. A sample size of 5 µl from the intact phenolics was injected for the HPLC analyses.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of bitter melon extracts (phenolics, ethanolic and aqueous) was determined according to the method of (Ruch et al., 1989). A solution of H2O2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (50 µg/ ml) in distilled water were added to a H2O2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contained the phosphate buffer without H2O2. The percentage of H2O2 scavenging of bitter melon extracts and standard compound (α- tocopherol) was calculated as:

$$\text{H2O2 scavenging effect (\%)} = \frac{control - sample}{control} \times 100$$

Where: $control$ is the absorbance of the control. $sample$ is the absorbaunce of the sample of extracts and standard.

Experimental design

Thirty adult male albino rats were used in this study and then divided as follow (each group consist six rats):

- **A.** Control group received standard pellet diet only for seven days and then killed.
- **B.** second group rat injected intraperitoneal with B. mallei at dose 10^6 cell/ ml.
- **C.** third group rat injected intraperitoneal with B. mallei at dose $1\,0^8$ cell/ ml.
- **D.** Fourth group rat injected intraperitoneal with B. mallei at dose $1\,0^6$ cell/ ml and treated with 250ug/ml of phenolic compounds for four weeks.
- **E.** Fifth group rat injected intraperitoneal with B. mallei at dose $1\,0^8$ cell/ ml and treated with 250ug/ml of phenolic compounds for four weeks.

Prepare of blood solution

The blood collects from rats by cardiac puncher, under anesthesia, and put in test tubes. After clotting, the tubes were centrifugation for 10 min to obtain sera. The serum was taken and stored by deep freezing until used.

III. MEASUREMENTS

AST, ALT and ALP

AST, ALT and ALP were measured by technique according to the instructions of manufacturer company kit (Randox).

Plasma Peroxidation levels (MDA), Glutathione (GSH) and Catalase

MDA (malonedialdehyied), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer (Abdul kareem et al., 2016). GSH level estimated bymixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer (Mahmood, 2010). Catalase was measured by using the procedure of Biovision-USA kits. All parameters were done on liver extract.

Statistical analysis

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one-way analysis of variance (ANOVA).

IV. RESULTS AND DISCUSSION

In this results the phenolic compounds of Equisetum arvense including silicic acid, isoquercitrin, apigenin, flavonoids and kaempferol that is in agreement with Badole, S. and Swati (Badole and Swati, 2014) that referred the phenolic compounds of Equisetum arvense including flavonoids, triterpenoids, isoquercetin, kaempferol, luteolin and apigenin. Scavenging activity of Equisetum arvense extracts (phenolics and aqueous) showed in

Table (1): Scavenging activity of Equisetum arvense extracts (phenolics and aqueous) and standard antioxidant compound (α- tocopherol) at concentration 50 µg/ml

Type of compound	H2O2 Scavenging activity (%)
α- tocopherol	42.5%
Aqueous extract	31.7%
Phenolic compounds extract	84.1%

In this results Phenolic compounds at 50 µg/ml, and aqueous extract exhibited 84.1% and 31.7 % scavenging activity of H2O2, respectively. that is in agreement with Al-Snafi (Tartik et al., 2015) that referred the Antioxidant activity of Phenolic compounds of Rheum ribes were estimated to be 98.13± 3.84.

1.Liver function tests

AST (109.82 ± 8.19; 175.65 ± 12.32 respectively), ALT (93.25 ± 9.49; 156.28 ± 14.34 respectively) and ALP (174.45 ± 19.43; 193.32 ± 12.49 respectively) in second and third groups show high significant increased ($P < 0.05$) compare with control group (13.42 ± 2.38, 17.39 ± 3.23 and 5365.18 ± 8.45 respectively). AST (14.72 ± 2.54), ALT (13.62 ± 1.54) and ALP (66.18 ± 6.43) in fourth group show no significant changes ($P < 0.05$) compared with control group. AST (41.72 ± 5.14), ALT (39.43 ± 7.39) and ALP (83.31 ± 9.06) in fifth group show significant increased ($P < 0.05$) compared with control group as shown in table (2). The infection by B. mallei lead to increase the levels of liver that back to the ability of B. mallei to induce hepatocytes degeneration with infiltration of mono-nucleated cells (Flayyih et al., 2013). The results of this study is in agreement with Khudhair et al. (Khudhair et al., 2014) that referred that phenolic and alkaloid compounds have potential effects om liver enzymes. Where after rats infected with Echinococcus granulosus, lead to increase the levels of liver enzymes but after treatment with phenolic and alkaloid compounds, liver enzymes back to normal ranges.

Table (2): The levels of AST, ALT and ALP in serum of the groups

Parameter Groups	AST (mg/dl)	ALT (mg/dl)	ALP (mg/dl)
Control	13.42 ± 2.38 d	17.39 ± 3.23 c	65.18 ± 8.45 c
Second	109.82 ± 8.19 b	93.25 ± 9.49 b	174.45 ± 19.43 b
Third	175.65 ± 12.32 a	156.28 ± 14.34 a	193.32 ± 12.49 a
Fourth	14.72 ± 2.54 d	13.62 ± 1.54 d	66.18 ± 6.43 d
Fifth	41.72 ± 5.14 c	39.43 ± 7.39 c	83.31 ± 9.06 c

Note: same letters mean non-significant changes and different letters mean significant changes

2.MDA, GSH and catalase in liver extract

MDA (2.12 ± 0.19; 2.95 ± 0.12 respectively), GSH (0.365 ± 0.043; 0.332 ± 0.056 respectively) catalase (0.84 ± 0.08; 0.72 ± 0.05 respectively) in rats exposure to X rays show high significant increased (MDA) and decreased (GSH) ($P < 0.05$) compared with control rats (1.87 ± 0.16; 0.476 ± 0.014 and 1.45 ± 0.21 respectively). MDA (1.81 ± 0.09; 1.93 ± 0.22 respectively) and GSH (0.462 ± 0.032; 0.469 ± 0.062 respectively) and catalase (1.48 ± 0.18; 1.33 ± 0.15 respectively) in show no significant changes ($P < 0.05$) compared with control rats as shown in table (3). In this study, the infection by B. mallei lead to increase the levels MDA, GSH and catalase but after treatment with phenolic and alkaloid compounds. Many studies have reported the advantages of phenolic compounds as antioxidant. In addition, there are relevant antioxidant enzymes to counter oxidants (Moo-Huchin et al., 2015; Lin et al., 2016). different bacterial species exhibit different antimicrobial sensitivities towards the tested phenolic compounds. Generally, the Gram-negative bacteria were observed to have more antimicrobial susceptibility than Gram-positive (Tyagi et al., 2015).

Table (3): The levels of MDA, GSH and catalase in liver extract of the groups

Parameters Groups	MDA (mmol/l)	GSH (mol/l)	Catalase (mol/l)
Control	1.87 ± 0.16 c	0.476 ± 0.014 a	1.45 ± 0.21 a
Second	2.12 ± 0.19 a	0.365 ± 0.043 c	0.84 ± 0.08 c
Third	2.95 ± 0.12 b	0.332 ± 0.056 b	0.72 ± 0.05 b
Fourth	1.81 ± 0.09 c	0.462 ± 0.032 a	1.48 ± 0.18 a
Fifth	1.93 ± 0.22 c	0.469 ± 0.062 a	1.33 ± 0.15 a

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