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Antioxidant Role Of Milk Thistle In Mice Infected With A Virulent Strain Of *Corynebacterium Pseudotuberculosis*

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Abstract
Present of this study was designed to improve the antioxidant activity of milk thistle (salymarin) against inoculated mice with virulent strain of *Corynebacterium Pseudotuberculosis* by measuring the serum levels of Free Radicals Peroxinitrite and Malondialdhyde. Fifty mice were divided into five groups equally G1:10 mice served as control negative. G2 served as control positive challenged virulent *C. pseudotuberculosis* (5×10^9) CFU/ ml I/P. G3 giving plant extract of salybum marianam 350 mg / kg orally. G4 giving saly marin for thirty days and challenged with *Corynebacterium pseudotuberculosis*. G5 challenged with virulent *C. pseudotuberculosis* (5×10^9) CFU/ ml I/P after thirty days treated with plant extract for thirty days. At thirty days of experiment (5) mice were sacrefecied from each group and serum blood were collected for measuring oxidant levels (Peroxinitrite and Malondialdhyde), also at sixty days of experiment all animal were sacrificed to determine the free radicals level (Peroxinitrite and Malondialdhyde). The results revealed that the serum levels of free radicals in group treated with milk thistle lower than serum levels treated with *Corynebacterium psedotuberculosis*. This study concludes that the antioxidant activity of milk thistle in prevent and treatment the oxidative stress.

Keywords: Milk thistle, *Corynebacterium psedotuberculosis* and oxidative stress.

INTRODUCTION

Silybum marianum (Milk thistle) an important medicinal plant uses in treated hepatic disturbance. Most common active ingredient in these plants are flavonolignans, collection of them called as silymarin include a mixture of three isomer are silybin, silycristin and silydianin. Features of its therapeutic are due to have silymarin in their composition (Qavami *et al*., 2013). Milk thistle (*Silybum marianum*) a well-known and commonly used herb for treating hepatic diseases, and has its possible greater patient acceptability (Post-White *et al*., 2007; Ghosh *et al*., 2010) Milk thistle constituents and seeds extract are hepatoprotective and antioxidant (Bhattacharya, 2011). Silymarin has been used to treat toxin-induced liver diseases, also alcoholic liver disease, acute and chronic viral hepatitis (Abenavoli *et al*., 2010). Silymarin restrain hepatitis C virus (HCV) infection and also displays anti-inflammatory, antioxidant, and immunomodulatory activity that provise to its hepatoprotective action (Polyak *et al*., 2010).

It has also a very potent antioxidant, anti-fibrotic and anti-inflammatory fetures (Ghosh *et al*., 2010; Fried *et al*., 2012). Manjinder and Rajesh (2006); Vaknin *et al*., (2007), mentioned the efficacy of milk thistle in prevented cancer such as breast, cervical and prostate cancer both in vivo and in vitro animal models. Milk thistle and its active coponent in treatment of cancer include their ability to strengthen the common drugs used as chemotherapeutic, also an inhibit the resistance of multidrug associated with protein to assist therapeutic drugs of cancer because of their immunostimulatory action and protection efficacy (Wilasrusmee *et al*., 2002; Manjinder and Rajesh , 2006). Leaves of milk thistle used as an herbal supplement for treating liver disease and biliary disorder (Post-White *et al*., 2007). Vaknin *et al*., (2007), record the cytoprotectant effect of milk thistle, an anticarcinogenic and supportive liver damage treatment from the poisoning of Amanita phalloides by the active ingredient of milk thistle (Silybum marianum) strongest antioxidant in prevents and helps cancer.

Oxidative stress, characterized by the production an excess of free radicals, is the main aspect of all living systems which use oxygen to convert biochemical energy coming from nutrients into adenosine triphosphate (ATP). Free radicals, also named ROS, that induce oxidative damage to some cellular macromolecules, as, proteins, lipids and Deoxyribonucleic acid. Reactive Oxygen Species increased in serum concentration has been implicated in the pathogenesis of some, common diseases (Cacciapuoti, 2016). Agarwal *et al*., (2012), Oxidants caused over production reactive oxygen species (ROS) and nitrogen species (RNS) than the levels of antioxidant is named oxidative stress.

Caseous lymphadenitis (CLA) is important chronic disease of adult and small ruminants that caused by *Corynebacterium pseudotuberculosis,* an aerobic gram positive small curved facultative bacillus (Silva *et al*., 2017). Also, *Corynebacterium pseudotuberculosis* cause ulcerative lymphangitis in horse (Rafael *et al*., 2017), and necrotizing granulomatous lymphadenitis in human as well as abscesses in the liver and internal lymph nodes (Abdullah *et al*., 2017). Abdullah *et al.,* (2013) and Moussa *et al*., (2016), mentioned that CLA disease was taken two forms an external form characterized by presence of abscesses in the superficially lymph nodes and subcutaneous tissue and the second form was internal form in which the internal organs were suffering from abscesses particularly lung, liver, kidney in addition to mediastinal and bronchial lymph nodes . Infected animals play important role in the spreading the infection

through contaminated soil, water, feed, and pastures by their feces, pus from rupture external abscess and respiratory secretion (Zaid *et al*., 2016). The important virulence factors of these pathogen are toxic lipid cell wall that play essential role in resistance of these bacteria to killing by phagocytic cells (Hard, 1975), the second type of virulence factor is sphingomyelin–degrading phospholipase D (PLD) exotoxin (Hodgson *et al.,* 1994). These factors cause increase vascular permeability that facilities bacterial dissemination within the host tissue (Batey, 1986; Windsor, 2011). This pathogen can invade the phagocytic cells and they can intracellular replication, survival and destroyed the macrophage (Yeruham *et al*., 2003).

MATERIALS AND METHODS

Preparation of plant extracts of milk thistle leave: According to Harborne *et al.,* (1975); Shivananda (2006), the methanolic extract of plant was done.

Determination of LD_{50} of plant extract of milk thistle: The procedure employed for the determination of LD_{50} was that described by Dixon (1980).

Bacterial strain:

***Corynebacterium pseudotuberculosis*:** Isolated the strain from a case of CLA in sheep. The strain was diagnosed previously by bacteriological method and PCR assay by Al Badrrawi (Al Badrrawi, 2016).

Preparation of *Corynebacterium pseudotuberculosis* infective dose (challenge): Challenge dose was prepared from the bacterial suspension of *C. pseudotuberculosis* according to (Khuder *et al*., 2012). Calculation and adjustment of this dose to 5×109 CFU/ml according to (Miles and Misra, 1938).

Determenation of serum malondialdehyde (MDA) concentration µM/L:

The concentration of serum MDA was measured by thiobarbituric acid (TBA) assay according to (Gilbert *et al*., 1984).

Determenation of serum peroxynitrite radical concentration µM/L:

Peroxynitrite ONOO convert to form nitrophenol was absorbed at 412 nm. The amount of nitrophenol that form in the serum which reflecting the level of peroxynitrite according to (van uffelen *et al*., 1998).

Experimental Design:

Fifty (BALB/ C Strain) mice and were used in this study, aged 8-10 weeks and weighed 25-30 gm were divided into five groups equally, each group, treated as the following.

***G1** (n=10): Giving normal saline orally 0.3 ml and served as a control group.

***G2** (n=10): Challenged with ($5x10^9$ CFU/ml) of virulent *C. pseudotuberculosis*.

***G3** (n=10): Administrated orally with plant extract milk thistle (350mg /kg bw) for thirty days.

***G4** (n=10): Challenged with ($5x10^9$ CFU/ml) of virulent *C. pseudotuberculosis*, after thirty day treated with milk thistle (350mg /kg bw) for thirty days.

***G5** (n=10): Administrated orally with plant extract milk thistle (350mg /kg bw) for thirty days and infected with virulent *C. pseudotuberculosis*. The period of this study was 60 days after first 30 days five mice from each group were sacrificed and serum blood were collected to determine the levels of free radical (Peroxinitrite and Malondialdhyde) in each group. Also, after the second 30 days (60 days of experiment) the process of blood sample collecting and measuring levels of oxidative stress were done.

RESULTS AND DISCUSSIONS

The results showed that serum peroxinitrite levels in G2 at 30 and 60 days were higher (**2.70± 0.009 and 3.10± 0.009) respectively than the serum values of G3 and G1 (0.31±0.007 and 0.27± 0.005) , (0.37± 0.002, 0.38±0.005) respectively . Also** serum peroxinitrite levels in G4 at 60 days when treated with milk thistle was lower (**0.28± 0.002) than values at 30 days of same group when** infected with C. pseudotuberculosis **(2.50± 0.001) and the serum values of G5 at 30 and 60 days after treated with milk thistle extract for sixty days and infected with** C. pseudotuberculosis at 30 days of experiment(**0.30± 0.002, 0.29± 0.009) respectively lower than G2 (2.70± 0.009, 3.10± 0.009) (table: 1).**

Table 1. Mean values of serum peroxynitrite at thirty and sixty days of experiment.

Time \ Group	Peroxynitrite at 30 days (µM/L)	Peroxynitrite at 60 days (µM/L)
G1	0.37± 0.002 C	0.38±0.005 C
G2	2.70± 0.009 AB	3.10± 0.009 A
G3	0.31±0.007 CD	0.27± 0.005 D
G4	2.50± 0.001 B	0.28± 0.002 D
G5	0.30± 0.002 CD	0.29± 0.009 D

Different capital letter means significant at ($P \leq 0.05$).

The results showed that serum **Malondialdhyde** levels in G2 at 30 and 60 days were higher (**2.80± 0.007and 3.20± 0.040) respectively than the serum values of G3 and G1 (0.39±0.008and 0.34± 0.006), (0.43± 0.007, 0.45±0.009) respectively. Also** serum peroxinitrite levels in G4 at 60 days when treated with milk thistle was lower (**0.41± 0.005) than values at 30 days of same group when** infected with *C. pseudotuberculosis* **(2.70± 0.004) and the serum values of G5 at 30 and 60 days after treated with milk thistle extract for sixty days and infected with** *C. pseudotuberculosis* at 30 days of experiment(**0.39± 0.002, 040.± 0.009) respectively lower than G2 (2.80± 0.007, 3.20± 0.040) (table: 2).**

Table 2. Mean values of serum peroxynitrite at thirty and sixty days of experiment.

Time \ Groups	Malondialdhyde at 30 days (µM/L)	Malondialdhyde at 60 days (µM/L)
G1	0.43± 0.007 C	0.45±0.009 C
G2	2.80± 0.007 B	3.20± 0.040 A
G3	0.39±0.008 D	0.34± 0.006 E
G4	2.70± 0.004 B	0.41± 0.005 C
G5	0.39± 0.002 D	040.± 0.009 C

Different capital letter means significant at ($P \leq 0.05$).

DISCUSSION

Present results revealed that the serum levels of MDA and peroxynitrate were high in infected animals (G2) at 30 and 60 days post infection as comparing with those levels in non-infected animals (G1) and treated animal with plant extract (G3 and G5) for both times, also G4 at 60 days when treated with plant extract after infected by *C.psudotuberculosis* these result may revealed that the infection with *C.psudotuberculosis* mediated oxidative stress due to these molecules were considered important markers of oxidative stress that cause DNA damage and lipid peroxidation (Ragab *et al*., 2013; Sırma *et al*., 2016). This evidence was agreement with Pacheco *et al*., (2012) who recorded that NO can response to extracellular protein of *C.psudotuberculosis* also these levels of peroxynitrate were highest in serum of non-immunized infected animals at 30 and 60 days post infection, these results may indicate that nitric oxide play important role in control infection of *C.psudotuberculosis*, this study was in consistence with Green *et al*., (1982), also Ogawa *et al*., (2001). who demonstrated that Nitric Oxide have a an important role in the coordinating growth of the intracellular bacteria. These free radical causes damage of cellular component such DNA that led to inhibit bacterial growth. Virulence pathogen such as *C.psudotuberculosis* can able to overcome intraphagosomal stress by changes of sigma factor genes (Helmann, 2002; Fontán *et al*., 2008), particularly sig E gene (Voskuil *et al*., 2011). The lower result of serum peroxinitrita and malondialdhyde in the groups treated with milk thistle (G3, G4 and G5) due to the antioxidant effect of this plant and this evidence a agreements with Mudit *et al*., (2010) who record that milk thistle have anti-inflammatory immunstimulatory and anti-oxidant features that led to the prevent of photocarcinogenesis in mice, also with Prague Medical Report, (2008), said that milk thistle cause decrease in liver enzymes activity specialty with (AST, ALT) act as antioxidant natural product for treatment liver toxicity. Results indicate that the porospect antioxidant activity of poly phenolic compounds (milk thistle) may be related to regulate ROS caused by free radicals in tumors (Feng *et al*., 2007; Viktorova *et al*., 2019). Flavonoids are generally thought to be having free radical scavenging and antioxidant effects (De Sampaio *et al.,* 2010). Also have neuroprotective actions (Spencer *et al.,* 2003; Zhong *et al*., 2017). milk thistle had a neuroprotective effect on microglial cell cultures and appears to inhibit nitric oxide production and iNOS gene activation (Kang *et al*., 2002). Agreement with Wang *et al*., (2002) who stated that neuroprotective role and neurotropic activities of silybin/silymarin (milk thistle) was due to its antioxidative activity. Milk thistle is an antioxidant and free radical scavenger (Muriel and Mourelle,1990). Milk thistle, due to their antioxidant work, has been found for preventing a rise in both pancreatic lipid peroxidation and plasma glucose in rats with hyperglycemic. Similarly, hyperplasia of islet of langerhans were reported in mice treated with alcohol after treatment with methanolic extract milk thistle (Al-taee *et al*., 2009).

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