**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 (silymarin) against inoculated mice with virulent strain of Corynebacterium Pseudotuberculosis by measuring the serum levels of Free Radicals Peroxinitrite and Malondialdehyde. Fifty mice were divided into five groups equally G1:10 mice served as control negative. G2 served as control positive challenged virulent C. pseudotuberculosis (5×10^7) CFU/ml I/P. G3 giving plant extract of silybum marianam 350 mg / kg orally. G4 giving salicylaryn 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 sacrificed from each group and serum blood were collected for measuring oxidant levels (Peroxinitrite and Malondialdehyde ), also at sixty days of experiment all animal were sacrificed to determine the free radicals level (Peroxinitrite and Malondialdehyde). The results revealed that the serum levels of free radicals in group treated with milk thistle lower than serum levels treated with Corynebacterium pseudotuberculosis. This study concludes that the antioxidant activity of milk thistle in prevent and treatment the oxidative stress.

Keywords: Milk thistle, Corynebacterium pseudotuberculosis 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, silicystin 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 promise 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 chemotherapy, also an inhibit the resistance of multidrug associated with protein to assist therapeutic drugs of cancer because of their immunostimulatory action and protection efficacy (Wilarsusmeec 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.

Casseous 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.
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through contaminated soil, water, feed, and pastures by their feces, pus from ruptured abscesses 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 (Hodison et al., 1994). These factors cause increase vascular permeability that facilitates bacterial dissemination within the host tissue (Batay, 1986; Windsor, 2011). This pathogen can invade the phagocytic cells and they can intracellular replication, survival and destroyed the macrophage (Yerutham et al., 2003).

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

Determination of LD50 of plant extract of milk thistle: The procedure employed for the determination of LD50 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 Badrassi (Al Badrassi, 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 5×105 CFU/ml according to (Miles and Misra, 1938).

Determination 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).

Determination 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 effelen 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 (5×106 CFU/ml) of virulent C. pseudotuberculosis.
*G3 (n=10): Administered orally with plant extract milk thistle (350mg /kg bw) for thirty days.
*G4 (n=10): Challenged with (5×106 CFU/ml) of virulent C. pseudotuberculosis, after thirty day treated with milk thistle (350mg /kg bw) for thirty days.
*G5 (n=10): Administered 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 (Peroxynitrite and Malondialdehyde) 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 peroxynitrite 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 peroxynitrite 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.

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

Different capital letter means significant at (P≤0.05).

The results showed that serum Malondialdehyde levels in G2 at 30 and 60 days were higher (2.80± 0.007 and 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 peroxynitrite 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, 0.40± 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.

<table>
<thead>
<tr>
<th>Time / Groups</th>
<th>Malondialdehyde at 30 days (µM/L)</th>
<th>Malondialdehyde at 60 days (µM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.43± 0.007 C</td>
<td>0.45±0.009 C</td>
</tr>
<tr>
<td>G2</td>
<td>2.80± 0.007 B</td>
<td>3.20± 0.040 A</td>
</tr>
<tr>
<td>G3</td>
<td>0.39±0.008 D</td>
<td>0.34± 0.006 E</td>
</tr>
<tr>
<td>G4</td>
<td>2.70± 0.004 B</td>
<td>0.41± 0.005 C</td>
</tr>
<tr>
<td>G5</td>
<td>0.39± 0.002 D</td>
<td>0.40± 0.009 C</td>
</tr>
</tbody>
</table>

Different capital letter means significant at (P≤0.05).
DISCUSSION

Present results revealed that the serum levels of MDA and peroxynitrite 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. pseudotuberculosis these result may revealed that the infection with C. pseudotuberculosis 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; Srma et al., 2016). This evidence was agreement with Pacheco et al., (2012) who recorded that NO can response to extracellular protein of C. pseudotuberculosis also these levels of peroxynitrite 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. pseudotuberculosis, 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. pseudotuberculosis 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 peroxinitrite and malondialdehyde 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 immunostimulatory and antioxidant 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 specially 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; Viktormva et al., 2019). Flavonoids are generally thought to have being 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 hyperglycemia. Similarly, hyperplasia of islet of langerhans were reported in mice treated with alcohol after treatment with methanolic extract milk thistle (Al-tae et al., 2009).

REFERENCES


