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The Antioxidant Activity of *Pinus merkusii* Ethanol Extract as Hepatoprotector in Mice

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Abstract

This study was carried out to investigate the activity of the extract of *Pinus merkusii* in protecting against lead acetate-induced hepatotoxicity. Mice were given *Pinus merkusii* 200 mg, 400 mg, and 800 mg/kg BW orally once in a day for 40 days, and on the 5th day, were given lead acetate 30 mg/kg BW one hour after *Pinus merkusii* extract administration. *Pinus merkusii* extracts significantly decreased the aspartate transaminase (AST), alanine transaminase (ALT), Malondialdehyde (MDA) levels and protected liver cell damage in lead acetate induced-hepatocyte. *Pinus merkusii* extract is a potent antioxidant and provide a promising hepatoprotective effect against lead acetate-induced hepatotoxicity.

Key words: *Pinus merkusii* extracts, MDA, lead acetate, hepatotoxicity

Several researchers demonstrated that oxidative stress is believed as one of the important mechanisms for lead acetate induced hepatotoxicity (Kaushik *et al.*, 2017). Oxidative stress is usually occurred due to a decrease in the antioxidant tissue defenses and the increasing production of reactive oxygen species (ROS) which will be indicated by elevated MDA (Xu *et al.*, 2008). The lead acetate also increased AST and ALT in liver damage (Wardani *et al.*, 2017). The *Pinus merkusii* has a potent antioxidant activity (Li *et al.*, 2015). Therefore this study was carried out to investigate the antioxidant activity of an extract of *Pinus merkusii* in protecting against lead acetate induced hepatotoxicity.

Materials and Methods

The forty male Balb/c mice were divided into 5 groups with group of 8 mice each: negative

control (mice were given aquadest daily) for 40 days, positive control (mice were given aquadest daily 5 days and on the 5th day, were given lead acetate 30 mg/kg BW orally once a day for 35 days), and the treatment group (mice were given *Pinus merkusii* extract 200 mg/kg BW, 400 mg/kg BW, 800 mg/kg BW orally once a day for 40 days, and on the 5th day, were given lead acetate 30 mg/kg BW one hour after *Pinus merkusii* extract administration for 40 days). On day 40, the blood sample was taken to measure the level of AST, ALT, and MDA, and liver tissues were fixed in formalin 10% to evaluate the histopathological study.

Results and Discussion

It has been reported that the lead acetate increased AST, ALT and MDA during liver damage (Wardani *et al.*, 2017). The *Pinus merkusii* has a potent antioxidant activity (Li *et al.*, *loc. cit.*). Analysis of these hepatic enzymes and MDA has carried out to evaluate the hepatoprotective effect of *Pinus merkusii* extract in the lead acetate treated mice. The administration of *Pinus merkusii* decreased AST, ALT and MDA level in lead acetate induced hepatotoxicity (Table I).

The lead hepatotoxicity causes oxidative stress due to imbalance between the generation of reactive oxygen species (ROS) and the scavenging capacity of antioxidants in the liver. Malondialdehyde (MDA), one of the well known secondary products of lipid peroxidation after exposure to ROS which is used as an indicator of cell membrane injury. The increase in serum MDA levels in liver damage suggests enhanced lipid peroxidation (Sudjarwo *et al.*, 2019). The antioxidant protective mechanism decreases the oxidative stress and scavenges the free radicals which are responsible for liver damage

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Table 1: Effects of *Pinus merkusii* extract on the AST, ALT and MDA in lead acetate induced hepatotoxicity

Groups	AST (U/L)	ALT(U/L)	MDA (nmol/ml)
Negative Control	66.30 ^a ± 3.27	27.70 ^a ± 2.75	4.97 ^a ± 0.86
Positive Control	92.10 ^b ± 5.59	43.40 ^b ± 2.63	8.04 ^b ± 0.86
<i>Pinus merkusii</i> 200 mg/kg BW	90.00 ^b ± 4.55	45.30 ^b ± 2.21	8.34 ^b ± 0.80
<i>Pinus merkusii</i> 400 mg/kg BW	85.80 ^a ± 4.77	42.20 ^b ± 2.15	7.84 ^b ± 0.79
<i>Pinus merkusii</i> 800 mg/kg BW	77.80 ^a ± 5.51	39.80 ^a ± 2.15	7.02 ^c ± 0.72

^{a,b,c}Different superscript within each column indicate significant difference between the means ($P < 0.05$)

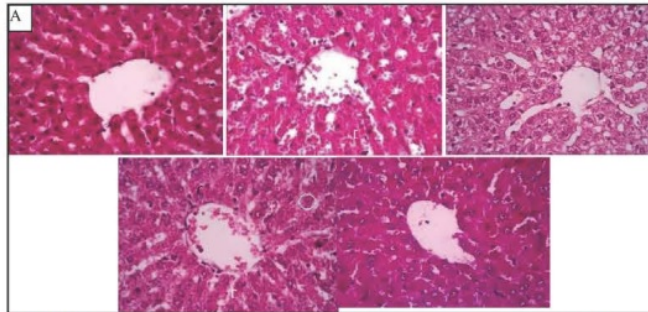


Fig 1: Histological study of *Pinus merkusii* extract on lead acetate-induced hepatotoxicity. The negative control (A); the positive control (B); Treated with *Pinus merkusii* extract 200 mg/kg BW, 400 mg/kg BW and 800 mg/kg BW (C, D and E). H&E (×400).

and thus inhibit the lipid peroxidation (MDA). In the study, *Pinus merkusii* extract, behaved an antioxidant and free radical scavenger, decreased the MDA level caused by lead acetate toxicity in mice. *Pinus merkusii* extract minimized the hepatotoxic effect of lead acetate through its antioxidant activity.

Liver damage following lead acetate exposure is well characterized by elevated levels of serum hepatic marker enzymes, which indicate cellular leakage and loss of functional integrity of hepatic membrane architecture (Wardani *et al., loc. cit*). The serum enzyme markers such as AST and ALT are recommended for the assessment of hepatocellular damage in preclinical studies as it is considered a more specific and sensitive indicator of liver damage (Kaushik *et al., loc. cit*). Low levels of AST and ALT are normally found in the blood, but when the liver is damaged or diseased, it releases more of AST and ALT into the bloodstream. Our results indicated that *Pinus merkusii* extract

lowered the AST and ALT levels. The findings of this study suggest that the antioxidant activity of ethanol extract of *Pinus merkusii* attenuate in lead acetate induced-hepatotoxicity.

Histologically the liver of mice in lead acetate treated group showed loss of the normal structure of hepatic cells, congestion, fatty degeneration and necrosis. Lead acetate thus had a deleterious effect on liver tissues. The treatment with *Pinus merkusii* extract dose-dependent inhibited the lead acetate induced liver damage (Fig 1).

It has been reported that the lead toxicity cause excessive production of ROS; there is an imbalance between the production of oxidants and the defense systems of antioxidant which may promote the induction of lipid peroxidation, proteins, and DNA damage, leading to hepatic cell death through apoptosis or necrosis (Sudjarwo *et al., loc. cit*).

Summary

The *Pinus merkusii* extract was given to the mice with induced lead acetate toxicity. The curative and protective effect of the extract on the liver and blood parameter was reported.

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Medical Management of Cellulitis of Limb in a Mule

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Abstract

A 15 year old mule was presented to the hospital with cellulitis of right hind leg. It had a history of sudden onset of swelling of right hind limb which started from fetlock joint and spread upto hip joint with lameness. The mule was not putting its weight on right hind limb and was preferring to lie down to avoid weight bearing on three limbs. Leukocytosis was observed on haematological examination and radiographic examination ruled out fracture. The mule was treated with broad spectrum antibiotics, steroids, NSAIDs, local dressing along with pressure bandage.

Key words: Cellulitis, Lameness, Leukocytosis

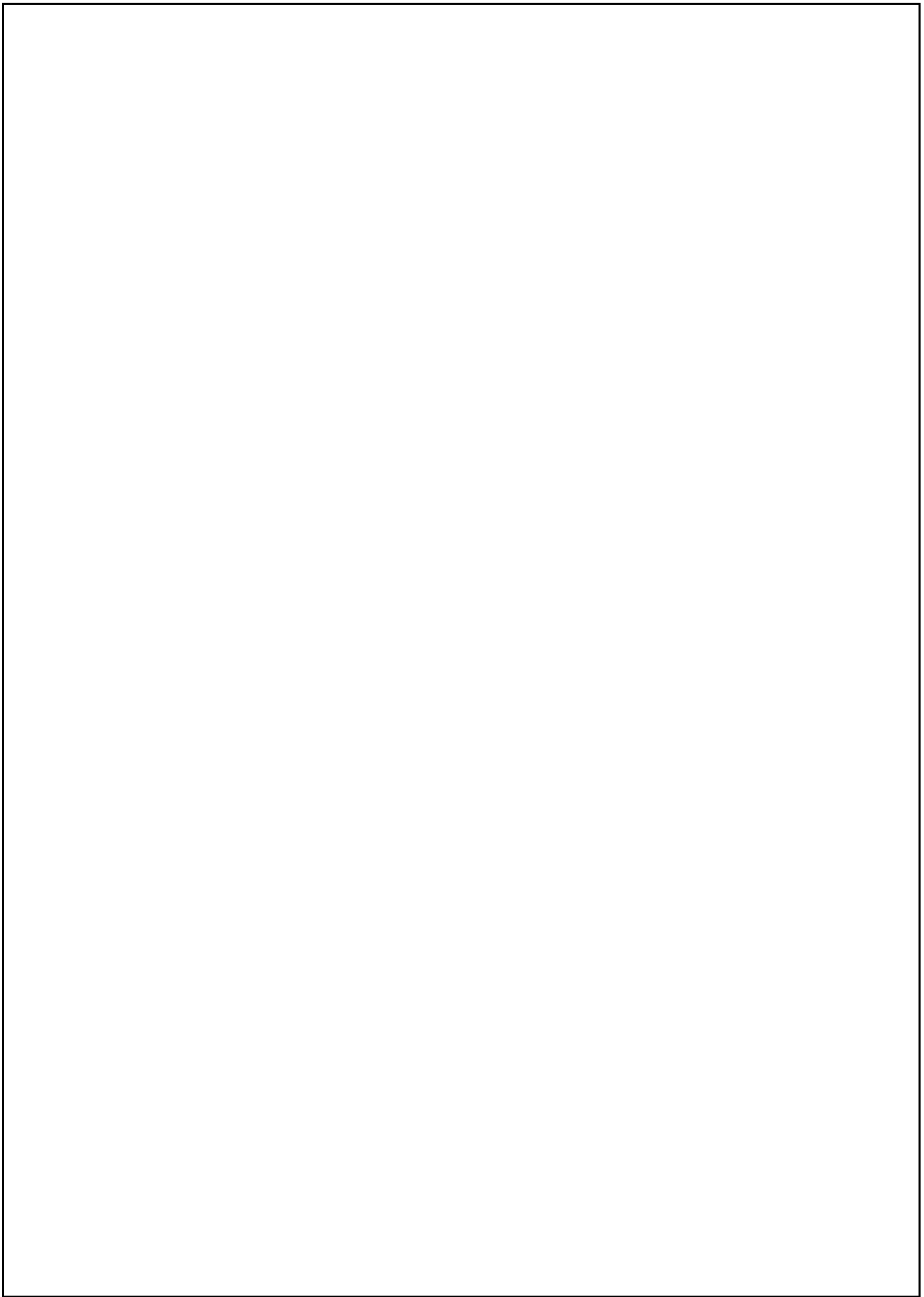
Diffuse soft tissue swelling affecting the limb, with prominent superficial lymphatic vessels is usually referred as cellulitis (Dyson, 2003), which is also known as lymphangitis, vasculitis, fat leg or big leg (Adam and Southwood, 2007). Cellulitis causing an acute painful

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swelling, often involving entire limb is frequently encountered in horses (Adam and Southwood, 2006). The underlying cause of cellulitis among horses is unknown, but sometimes associated with soft tissue trauma and subsequent bacterial infection (Adam and Southwood, 2004). Predisposing factors for the development of cellulitis in humans include vascular insufficiency, defective venous or lymphatic drainage, previous cellulitis, foreign body, diabetes mellitus, accidental or surgical trauma (Dinubile and Lipsky, 2004). Acute cellulitis cases are treated with broad spectrum antibiotics and NSAIDs along with supportive therapies.

Case History and Observations

A 15 year old mule weighing around 350 kg was presented to the hospital with a history of sudden onset of swelling of right hind limb which started from fetlock joint and spread upto hip joint along with lameness. The patient was evacuated to the hospital two days post onset of clinical signs. Initially mule was treated



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