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# Research Article
# Effect of Perennial Herb *Angelica keiskei* on Serum Hepatic Enzymes, Renal Function Test and Wound Size in Scalded Rats

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## Abstract

**Background and Objective:** Elevation of the serum hepatic enzymes increased the length of hospital stays in burn injury patients. This study was aimed to investigate the effect of the perennial herb, *A. keiskei* on levels of serum hepatic enzymes, renal function and wound size in scalded rats. **Materials and Methods:** Scald wound rat model was created by hot water on the dorsal surface. Rats in control groups were given a placebo, but the rats in treatment group were given *A. keiskei* extract (300 mg $kg^{-1}$ b.wt.) after the burn treatment for 14 days. Blood serum was analyzed for ALT, AST, ALP test on the 2nd, 8th and 14th day. The burnt wound size was measured on the 1st, 5th, 8th, 12th, 15th and 18th day. For statistical analysis, the Wilcoxon signed rank test to analyze the levels of ALT, AST, ALP, urea and creatinine in serum. The wound sizes between the groups were analyzed with Mann Whitney U Test. **Results:** In the control group, the levels of ALT and AST showed no statistical differences. However, in the treatment group, the levels of ALT on 8th and 14th day and levels of AST on 8th day were decreased. The levels of ALP in the control group were increased on 8th and 14th day, in the treatment group, the levels of ALP on the 8th day decreased significantly. Levels of creatine and urea serum in the treatment group showed no significant difference. Statistic differences of wound sizes, between control and treatment groups were significant on 15th and 18th day. **Conclusion:** *Angelica keiskei* extract decreased the levels of serum ALT, AST, ALP and scald wound size in scalded rats.

Key words: Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase wound size, *Angelica keiskei*, scalded rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Scalded wounds are wet burn injuries on skin result from exposure to high-temperature water such as boiling water[1]. According to WHO (2018), burns are a global public health problem, the majority of these occur in low- and middle-income countries and almost two-thirds occur in the WHO African and South-East Asia regions[1]. The systemic response to burn is often accompanied by secondary damage to vital organs such as liver and kidneys which are distant from the injured skin[2-4]. Hepatomegaly is common findings in thermal injuries, which lead to cell damage. Liver enzymes, such as AST and ALT are the most sensitive biochemical indicators of hepatocyte injury[5,6]. Cellular damage increases the cell membrane permeability, these enzymes leak into the bloodstream. ALT is a more sensitive and specific test for hepatocyte injury since AST also can be increased in the state of muscle injury. In burn injury, serum AST, ALT and ALP might be elevated between 50-200% from baseline when compared with normal levels[5,7].

In thermal injury, a balance between Reactive Oxygen Species (ROS) and antioxidants are disrupted. Inflammatory cells, including activated neutrophils, produce a burst of free radicals[8-10]. Supplementation of antioxidants in human and animal models has proven benefit in wound healing postburn injury. Recent studies reported the beneficial effect of antioxidants given to burn patients. The reduction in healing time was reported on burn injury patients that administered orally capsule antioxidants (vitamin E, vitamin C, zinc sulphate, allopurinol, melatonin and N-acetylcysteine)[8-10]. A lipophilic antioxidant, Coenzyme Q10 treatment attenuated burn injury-induced metabolic alterations (hyperlactatemia) and oxidative stress in mouse skeletal muscle[11]. Astaxanthin (a natural and powerful antioxidant)[12], decreased oxidative stress and apoptosis on acute kidney injury following burn injury in rats[13].

The antioxidant effect of a perennial herb, i.e., *Angelica keiskei* (*A. keiskei*) has been proven in numerous scientific journals due to the presence of bioactive constituents such as: flavonoids, coumarins, terpenoids, acetylenes and phenolics compounds[14-20]. *Angelica keiskei* significantly increased antioxidant enzymes, such as: hepatic catalase and glutathione transferase in rats fed a high-fat diet[21], Antioxidant effect of *A. keiskei* extract was achieved at nontoxic dosage 300 mg $kg^{-1}$ b.wt./day in Sprague Dawley rats[22], the present study, analyzed the changes of serum ALT, AST, ALP and wound size in scalded rats treated with *A. keiskei* extract.

## MATERIALS AND METHODS

**Study area:** The main scope area of this research is on biochemistry science, including hepatic enzymes and renal function tests. The decreased levels in the plasma of hepatic enzymes are indicators of the beneficial effects. A renal function test was done to know the side effect of the herb. This research was conducted from 7 September, 2019 to 3 February, 2020.

**Animal species selection:** Healthy, adult male Sprague-Dawley rats, weighing 180-200 g were used in the present study. They were housed in the animal house of the medical faculty at Diponegoro University. The animals were provided free access to water and food *ad libitum* and were adapted to temperature (27±2°C), humidity (50±5%) and 10-14 h of light and dark cycles for acclimatization.

**Thermal injury to rats:** The thermal injury was conducted with a method described by Zhang XG, *et al.*[23] with slight modifications. A total of 10 rats were randomized into 2 groups, each group consisted of 5 rats. All rats in both groups were shaved to obtain a hairless round area with a diameter of 5 cm. The rats were anesthetized by injecting 0.1 mL/100 mg of ketamine intraperitoneally and antiseptic povidone-iodine was applied. After 15 min, the shaved area was doused with 100 mL of boiling water for 30 sec through a cylinder with a diameter 2.5 cm circular opening. Rats in control groups were given a placebo, but the treatment groups were given *A. keiskei* extract 300 mg $kg^{-1}$ b.wt., after the burn treatment and once every 2 days for 18 days after the burn treatment. Blood serum was analyzed for ALT, AST, ALP test on the 2nd, 8th and 14th day. The wound size was scored by multiplying the largest diameter by its perpendicular to the middle diameter, measured with a millimeters ruler on the 1st, 5th, 8th, 12th, 15th and 18th day. This study was carried out after obtaining clearance from the institutional animal ethics committee Diponegoro Medical Research Institute, No. 102/EC/H/KEPK/FK-UNDIP/VII/201.

***Angelica keiskei* extract:** *Angelica keiskei* extract was, collected from Denpasar, Bali Indonesia Farm. It has been registered with certificate no: P-IRT: 213510801105 by Indonesia Public Health Office. Rats in control groups were given a placebo, but the treatment groups were given *A. keiskei* extract (300 mg $kg^{-1}$ b.wt.) after the burn treatment for 14 days.

**Statistical analysis:** Data were analyzed with SPSS 17.0, the levels of ALT, AST, ALP, urea and creatinine were analyzed with the Wilcoxon signed-rank test. While the sizes of scald wound area between the groups were analyzed with the Mann Whitney U Test.

## RESULTS

**Effects on the levels of serum ALT, AST and ALP:** The present study shows the hepatoprotective effects of *A. keiskei* extract in scalded rats that decreases serum levels of ALT, AST and ALP.

**Levels of serum ALT:** The findings showed that serum levels of ALT observed in samples taken on 2nd, 8th and 14th day were not statistically different in the control group, but in the treatment group, the serum levels of ALT on 8th and 14th day were statistically lower than 2nd day ($p = 0.049$) as seen in Table 1.

**Levels of serum AST:** The serum levels of AST observed in samples taken on 2nd, 8th and 14th day were not statistically different in the control group, but in the treatment group, the levels of AST on 8th day were statistically lower than 2nd day, $p = 0.028$ (Table 2).

**Levels of serum ALP:** The serum levels of ALP were observed in samples taken on 2nd, 8th and 14th days. The results showed that the levels of ALP significantly increased in the control group on the 8th and14th day, but the levels of ALP on the 8th day were statistically lower than 2nd day, $p = 0.028$ as resumed in Table 3.

**Effects on scald wound size:** In the present study, the healing effect of *A. keiskey* was observed by comparing scald wound sizes between control and treatment groups on 1st, 5th, 8th, 12th, 15th and 18th day. The wound sizes in the treatment group significantly smaller on the 15th day ($p = 0.047$) and 18th day ($p = 0.028$) as shown in Table 4.

**Effects on serum levels of creatinine and urea:** The serum levels of creatinine and urea were observed in samples taken on 2nd, 8th and 14th day. The findings showed no statistical difference ($p>0.05$) on the levels of serum creatinine both in the control group (Fig. 1) and treatment group (Fig. 2). The levels of serum urea in the control group (Fig. 3) and treatment group (Fig. 4) showed no statistical difference ($p>0.05$).

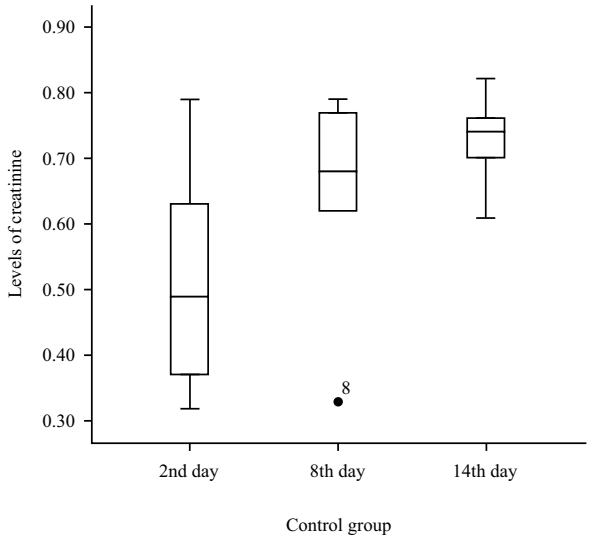


Fig. 1: Levels of creatinine in control group

Table 1: Effect of *A. keiskei* on levels of serum ALT in scalded rats

| | Levels of serum ALT (IU $L^{-1}$) | | |
|---|---|---|---|
| Groups | 2nd day | 8th day | 14th day |
| Control | 57.0±9.0 | 65.6±42.5 | 56.2±10.2 |
| Treatment | 75.0±19.8[a] | 50.8±6.5[b] | 61.2±16.1[b] |

Observed in samples taken on 2nd, 8th and 14th day, values are expressed as the Mean±SD, n = 5, different alphabets mean significantly statistic difference at $p<0.05$

Table 2: Effect of *A. keiskei* on levels of serum AST in scalded rats

| | Levels of serum AST (IU $L^{-1}$) | | |
|---|---|---|---|
| Groups | 2nd day | 8th day | 14th day |
| Control | 221.4±79.3 | 185.4±136.4 | 188.4±26.8 |
| Treatment | 232.4±39.6[a] | 143.6±30.1[b] | 194.6±44.0[a] |

Observed in samples taken on 2nd, 8th and 14th day, values are expressed as the Mean±SD, n = 5, different alphabets mean significantly statistic difference at $p<0.05$

Table 3: Effect of *A. keiskei* on levels of serum ALP in scalded rats

| | Levels of serum ALP (IU $L^{-1}$) | | |
|---|---|---|---|
| Groups | 2nd day | 8th day | 14th day |
| Control | 439.2±327.8 | 813.6±243.9* | 955.7±246.9* |
| Treatment | 232.4±39.6 | 143.6±30.1[#] | 194.6±44.0 |

Observed in samples taken on 2nd, 8th and 14th day, values are expressed as the Mean±SD, n = 5, *significant increased in the control group on 8th and 14th day, but in the treatment group, [#]Levels of ALP on 8th statistically lower than 2nd day

Table 4: Effect of *A. keiskei* on wound sizes in scalded rats

| | Diameters of wound size (cm) | | | | | |
|---|---|---|---|---|---|---|
| Groups | 1st day | 5th day | 8th day | 12th day | 15th day | 18th day |
| Control | 6.25±0.0 | 4.86±0.9 | 4.72±0.8 | 4.57±0.7 | 4.55±0.5* | 4.34±0.7# |
| Treatment | 6.25±0.0 | 4.45±0.7 | 4.19±0.8 | 3.81±0.6 | 3.75±0.7** | 3.00±0.6## |

Observed in samples taken on 1st, 5th, 8th, 12th,15th and 18th day, values are expressed as the Mean±SD, n = 5, **compared to the control group, the wound sizes in treatment groups were significantly smaller on 15th day and ##on 18th day

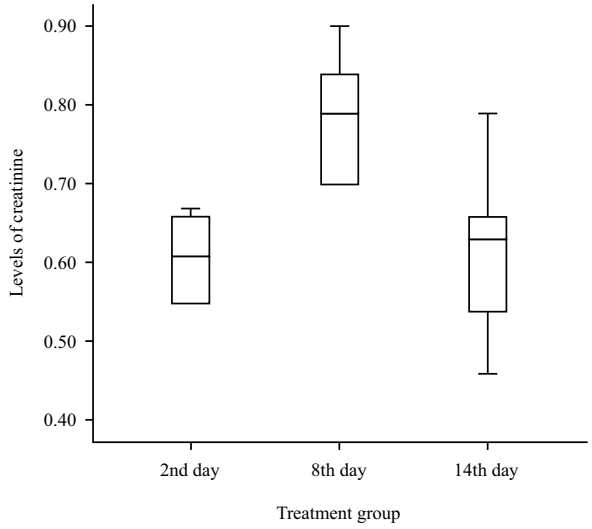


Fig. 2: Levels of creatinine in treatment group

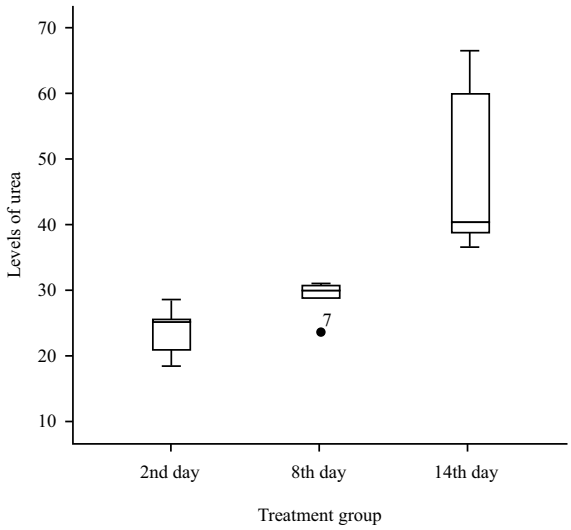


Fig. 4: Levels of serum urea in treatment group

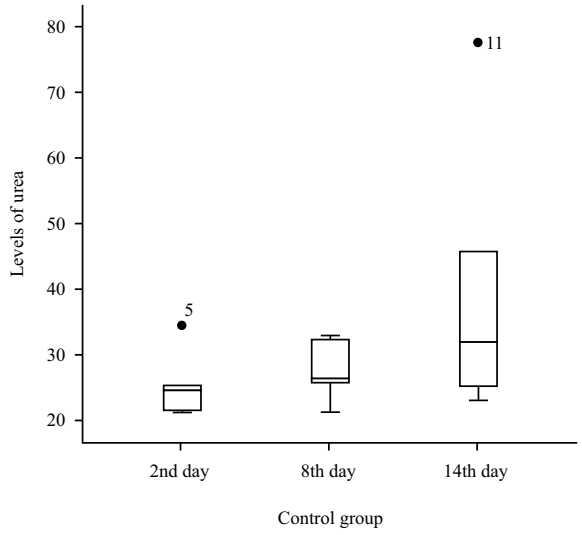


Fig. 3: Levels of serum urea in control group

## DISCUSSION

This study explored the beneficial effects of *A. keiskei* extract in thermal injury by measuring levels of liver enzymes, wound healing and levels of serum creatinine and urea. Decreased levels in the plasma of hepatic enzymes may be indicators of the beneficial effects because a previous study reported that elevation of the serum hepatic enzymes increased the length of hospital stays in burn injury patients with preexisting liver disease[23]. A thermal injury might be accompanied by systemic inflammatory reactions, multiple organ failure[24]. Inflammatory cells, activated neutrophils and macrophages produce ROS[10,11]. Increased ROS levels should be scavenged to $H_2O$ and $O_2$ by antioxidants. Antioxidative enzymes in liver, including superoxide dismutase (SOD), glutathione peroxidase and catalase are potent ROS scavengers but occur in cells only at relatively low concentrations. Oxidative-stress pops up whenever the balance between Reactive Oxygen Species (ROS) and antioxidants are disrupted[25-27]. Treatment with antioxidant *A. keiskei* extract in the present study was done to counteract the effects of ROS produced in thermal injury. Compared to the control group, the serum levels of ALT, AST and ALP decreased significantly in the treatment group.

Previously published studies reported the quicker healing scald wound effects by giving treatment with antioxidants in thermal injuries. The antioxidants found in *Callicarpa nudiflora* contained acteoside, flavonoids and triterpenoids significantly

accelerated the wound healing process and lowered the scald wound areas on 15th day[23]. Extract of Pheretima related to its antioxidation ability promoted healing of the wound surface in scalded rats[28]. Polyphenols in Argan oil produced from Argania spinosa[29] ameliorated burn-wound skin injury in rats[30]. In the present study, the healing effect of *A. keiskey* was observed by comparing scald wound sizes between control and treatment groups. The wound sizes decreased significantly on 15th day ($p = 0.047$) and 18th day ($p = 0.028$) in treatment group.

Renal function test was done to know the side effects of the herb to the kidney. Even though the previous study reported no significant differences in serum creatinine and urea levels at a high dosage of *A. keiskei* (1000 mg $kg^{-1}$ b.wt.) administered orally to Wistar rats[31-33], future studies should evaluate the optimal dosage of *A. keiskei* extracts for thermal injury. In the present study, a lower dosage of *A. keiskei* (300 mg $kg^{-1}$ BW) showed beneficial effects in scalded rats. The serum levels of creatinine and urea in the control group and treatment group showed no statistical difference ($p>0.05$).

## CONCLUSION

In thermal skin injury, inflammatory cells including activated neutrophils produce a burst of reactive oxygen radicals. Supplementation of perennial herb *A. keiskei* as potential antioxidants in animal models has proven benefit in wound healing. The findings of this study showed decreased levels in serum levels of hepatic enzymes are beneficial indicators of thermal injury. Healing effects were proven by decreased diameters of wound size. The results also prove the *A. keiskei* extract is not toxic to the kidney by analyzing the levels of urea and creatinine tests.

## SIGNIFICANCE STATEMENT

This study discovered the liver protective of *A. keiskey* extract in scalded injury. Decreased levels of ALT, AST and ALP are beneficial because the elevation of the serum hepatic enzymes worsened the prognosis in burn injury patients. This study will help researchers to uncover the critical unexplored healing effects of *A. keiskei* without toxic effect to the kidney.

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