Original Article

# Anti-Ageing Effect of *Pandanus Tectorius'* Seed Extract on D-Galactose Induced Ageing Mouse Model

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## Abstract

The anti-ageing effect of Pandanus tectorius' seed extract (PTSE) on D-galactose induced ageing mice and its mechanism was examined in this study. The biochemical and histological analysis of skin tissues showed that PTSE could effectively improve the antioxidant enzyme activity of the ageing mice. In addition, it could enhance the activity of superoxide dismutase (SOD) and decrease the malondialdehyde (MDA) content. Besides, PTSE could maintain the skin collagen, and its effect was found not less than that of the well-known vitamin C. The therapeutic properties of PTSE may possibly be attributed to the phenolic compound present in it. Overall, these results seem to be implying that PTSE is a potential natural anti-skin ageing agent with great antioxidant ability.

**Keyword:** superoxide dismutase, malondialdehyde, *Pandanus tectorius,* skin collagen, phenolic compound

## Introduction

Skin ageing is a complex natural process influenced by two mechanisms i.e. intrinsic ageing (genetic, chronological) and extrinsic ageing (photoageing).[1] Both mechanisms are associated with changes in the physical, morphological and physiological properties of the epidermis and dermis.[2] The two processes overlap and are strongly associated with an increase in free radicals and phenomenon of oxidative stress in the skin.[3] One of the main factors accelerating intrinsic skin ageing is oxidative damage to cellular structures. To counteract the changes resulting from oxidative stress, the body has developed many mechanisms to protect against the generation of free radicals and to convert them into inactive derivatives. These mechanisms include compounds of both exogenous and endogenous origin that form a complex antioxidant system. The enzymatic antioxidants include superoxide dismutase (SOD), vitamin C and polyphenolic compound.[4] Among the causative factors of skin ageing, repetitive sunlight exposure has been considered as the most common factor.[5] Repetitive UV exposure can cause skin ageing and can lead to the accumulation of peroxyl free radicals caused by the breakdown of malon dialdehyde (MDA). These events cause further decrease in skin elasticity and its water holding capacity, which is a common symptom of photo-aged wrinkling skin.[6] Thus, one of the ways to prevent the ageing effect on skin is by scavenging the free radical formation in the skin by using antioxidative agents. The ageing animal model induced by D-galactose (D-gal) [7] is considered as a natural model of ageing. Compared with other ageing animal models, the one that is induced with D-galactose is simple, inexpensive and stable.[8] D-gal animal model has become an internationally recognized model and has been widely used in the field of anti-ageing medicines. In addition, free radicals generated from D-gal metabolism in vivo can result in ageing as well.[9] Therefore, using D-gal to build the body or the skin ageing model is both reasonable and effective. Synthetic antioxidants are used in cosmetics and pharmaceuticals to inhibit reactive oxygen species (ROS). However, their long-term use is known to exert toxic

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: 10.4103/jnsbm.JNSBM_13_2_9

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**Submitted:** 05th December, 2022 **Received:** 09th December, 2022
**Accepted:** 22nd December, 2022 **Published:** 08th January, 2023



**How to cite this article:** Rahayu S E, Arika L A L, Anisa P P, Suprihatin. Anti-Ageing Effect of *Pandanus Tectorius'* Seed Extract on D-Galactose Induced Ageing Mouse Model. J Nat Sc Biol Med 2022;13:147-152

effects. Though, many studies have discovered more effective and much safer natural antioxidants.[10] Plant extracts and their bioactive metabolites have proven to be effective against UV radiation.[11] For example, *Pandanus tectorius (P. tectorius)* has many biological activities such as antioxidant[12], anti-inflammatory[13], antibacterial[14], cytotoxic[15] and anti-atherosleroisis[16]. Amongst other parts,, seeds of this plant have the highest antioxidant properties.[14] It is reported that seed extract of *P. tectorius* contains many phytoconstituents, such as phenol, flavonoid, steroids, terpenoids and saponin.[12] In the present study, anti-ageing and antioxidant effects of *P. tectorius'* seed extract in D-gal induced skin ageing mouse model were examined.

## Materials and Methods

### Seed Samples

Seeds of *P. tectorius* were collected from Ujung Genteng, Sukabumi, West Java, Indonesia. The seeds were collected from mature fruit of the plant which was shown by the orange color of its exoderm. The seeds were shade dried and mechanically ground to a powder form. About 1000 g of this powder was cold macerated using 98% methanol for 15 days, while changing the solvent every 5 days. The collected solvent was filtered with whatman filter paper and then evaporated at 40°C under low pressure until a semi-solid residue was obtained. The phenolic and flavonoid contents of PTSE consisted of 118.17mg GAE/g (Gallic acid equivalent/g of PTSE) and 8.5mg QE/g (Quercetin equivalent/g of PTSE). Moreover, the IC50 values of DPPH scavenging effect were 41µg/mL according to our previous investigations. Later, the PTSE was stockpiled at -20°C.

### Animals and Treatment

Approval of all experiments was obtained by the Institutional Health Research Ethics Committee of Universitas Pembangunan Nasional Veteran Jakarta. Mice aged 12 weeks, weighing around 25.0± 3.0g, were obtained from Faculty of Animal Husbandry of Agricultural University Bogor, Indonesia. Throughout the experiment, animals were housed in an air-conditioned room at a temperature of 25-27°C under daylight cycle of 12h. The animals were acclimatized for 7 days prior to the experiment. During the whole experiment, the performance and body weight of mice were recorded daily. In deferring organism-ageing experiment, all mice were split into six groups at random; each group comprised six male mice. The model group (ageing control + distilled water), control group, vitamin C group (VC), low dose, medium dose and high dose PTSE groups. All the groups were subcutaneously injected with D-gal prepared in normal saline, once daily at a dose of 500 mg/kg for 42 days. While, the mice in control group were treated with distilled water in the same volume. After injection of D-gal, the VC group received Vitamin C (100 mg/kg per day) and the low, medium and high dose PTSE groups received PTSE at doses of 75 mg/kg, 150 mg/kg and 225 mg/kg per day, respectively. Meanwhile, the model and control groups were orally administered with distilled water for 42 days. After the last treatment, mice were sacrificed and their blood and tissues were immediately collected and homogenized (4°C, 3000 rpm for 10 min) for biochemical and histological analyses.

### Biochemical and Histological Analysis

The levels of SOD and MDA in blood were determined using relevant commercial kits. All procedures were performed according to the manufacturer's instructions. After being fixed in 10% formalin for 24h, tissues and dorsal skin samples were progressively dehydrated in different concentrations of ethanol, hyalinized in xylene, embedded in paraffin, sliced into thin section (5 µm), dewaxed and colored with hematoxylin-eosin (HE). Cross sections were selected from three plates per sample. The thickness of collagen was measured by using a digimatic caliper.

### Statistical Analysis

The obtained data was statistically analyzed using the SPSS program version 26 for windows and the differences were considered significant at $p<0.05$. One-way Analysis of variance (ANOVA) was used to compare the data between each sample.

## Results

### 3.1 Effect of PTSE on the MDA Content in Skin Ageing Mice

As shown in Table 1, the MDA content of model group was significantly higher than that of the control group ($p<0.05$) which illustrated the successful set-up of ageing model. The MDA content of VC and high dose PTSE groups were lesser than that of model group. The MDA of skin ageing mice was effectively reduced with oral administration of high dose of PTSE. In the deferring skin-ageing experiment, the MDA content of model group was significantly higher than that of control group, VC group and PTSE groups as shown in Table 1. The MDA levels of high dose PTSE group were much less than that of model group which depicted that the content of MDA in the skin ageing mice was reduced by PTSE in dose dependent manner.

**Table 1. Antioxidant Status in Blood of Mice in Each Group**

| Groups | MDA (nmol/mL) | SOD (µ/mL) |
|---|---|---|
| Control | 0.70±0.13* | 15.56±3.89 |
| Model | 0.91 ±0.09 | 25.60±5.31 |
| VC | 0.90±0.09 | 22.08±5.31 |
| Low dose PTSE | 1.21±0.09* | 36.89±10.35 |
| Medium dose PTSE | 0.98±0.13 | 37.38±8.78 |
| High dose PTSE | 0.86±0.11 | 30.94±8.84 |

Values are presented in mean ±SD

### Effect of PTSE on the SOD Activity in Skin Ageing Mice

As shown in Table 1, the SOD level in model group was higher than that in the control and VC groups, whereas it was found lower in this group than all the PTSE groups. However, VC group had no significant difference in SOD level when compared with PTSE groups. Moreover, no significant difference in SOD levels was found among the three groups of PTSE ($p<0.05$).

### Effect of PTSE on Physical Properties of Skin Ageing Mice

As shown in Table 2, the collagen density of mice in model group was lower than that in the control group. The collagen density fiber content in VC group was almost the same as in the model group, but lower than in control group. Whereas, its content in medium and high dose PTSE groups was higher than that in VC group (Figure 1(E) and 1 (F)).

**Table 2. Effect of PTSE on Collagen Density in Mice Skin**

| Group | Collagen density ($\mu g/mm^3$) |
|---|---|
| Control | 31.40±3.70 |
| Model | 27.47±2.72 |
| VC | 27.27±0.27 |
| Low dose | 27.83±0.76 |
| Medium dose | 36.30±0.14 |
| High dose | 39.74±12.67 |

Values are presented in mean±SD

## Discussion

The model group in all experiments showed obvious differences with other groups in daily behaviour, pathological sections and biochemical indexes. It is well documented that the environmental factors contribute to vulnerability of the skin which lead to its premature ageing.[17] The factors responsible for accelerated skin ageing are largely contributed by over-expression of ROS. Indeed, the skin ages due to the imbalance in the expression of free radicals.[17] As previously noted, free radicals from D-gal metabolism in vivo could result in ageing. Therefore, the level of ROS induced in the skin plays a central role in the responses towards premature skin ageing.

When ROS are generated, the intracellular oxidant levels increase which cause two potentially important effects i.e. damaging of various cell components and triggering of the activation of specific signalling pathways.[18] These effects may significantly affect a variety of physiological processes and metabolic pathways closely related to ageing of the skin. For instance, excess ROS can make lipid peroxidation in vivo, the final oxidation product of which is MDA which can direct the protein and nucleic acid to cross-link and become cytotox.[19] Therefore, the content of MDA may directly reflect the body's level of lipid peroxidation and indirectly the level of cell damage brought by ROS.

A complex antioxidant-defense system, including the major enzymatic scavengers i.e. SOD, can eliminate most of the adverse impact caused by ROS. The SOD can catalyze the rapid transformation of superoxide to hydrogen peroxide.[20] Also, it is the primary free radical scavenger in vivo whose sole function is the removal of superoxide anions. In addition, SOD activity is the visual indicator of the level of organism's ageing.[21]

The skin ageing process is closely dependent on various pathological and physiological processes. Amongst these, degradation of extracellular matrix biomolecules such as collagen is very important, because this protein is involved in maintaining the skin in good condition.[22] Collagen, a major component of the skin, is degraded by the enzyme collagenase. Due to the fact that inhibition of collagenase is one of the key factors that can prevent the loss of skin elasticity and thereby delay the ageing process, in the present study, the possibility of inhibiting this enzyme by PTSE has been examined.

The present study suggests that a substance with excellent free radical scavenging activity has a good anti-ageing effect. It was observed that the antioxidative effect of PTSE selectively preserved the activity of SOD and inhibited the formation of MDA under oxidative stress condition. Inhibiting the formation of ROS plays an important role in protecting the endogenous antioxidative system from oxidative stress.

According to this study, the oral administration of PTSE could effectively improve the antioxidant defense system of the ageing mice, enhance the activities of SOD and decrease the MDA content as well. Similarly, it also provided visible improvement in skin condition of the ageing mice, such as the increase in collagen density. Although it is well known that VC has a greater antioxidant activity in vitro and in vivo, PTSE was found to have even better antioxidant activity as shown in Figure 1. A study has reported that the damage resulting from ROS could lead to stepped-up consumption of collagen in the skin.[23] Collagen fiber (mainly collagen) is an important part of the dermis and with increasing age, its content decreases significantly which makes the skin saggy and inflexible. Therefore, the change in collagen content can prompt the degree of skin ageing. The current study found PTSE to be effective in improving the condition of mice skin. Moreover, both VC and PTSE were found to have similar skin-caring activity as both effectively enhanced the SOD activity, maintained collagen and reduced MDA content in the skin of ageing mice. Particularly, PTSE effectively decreased MDA content which illustrated its anti-lipid peroxidation activity. Our data suggested that PTSE delays skin ageing via strengthening the antioxidant capacity to some extent. Furthermore, its effect was not less than that of the well-known VC. These results seem to be implying that PTSE has great potential for developing anti-ageing medicines and skin care products.

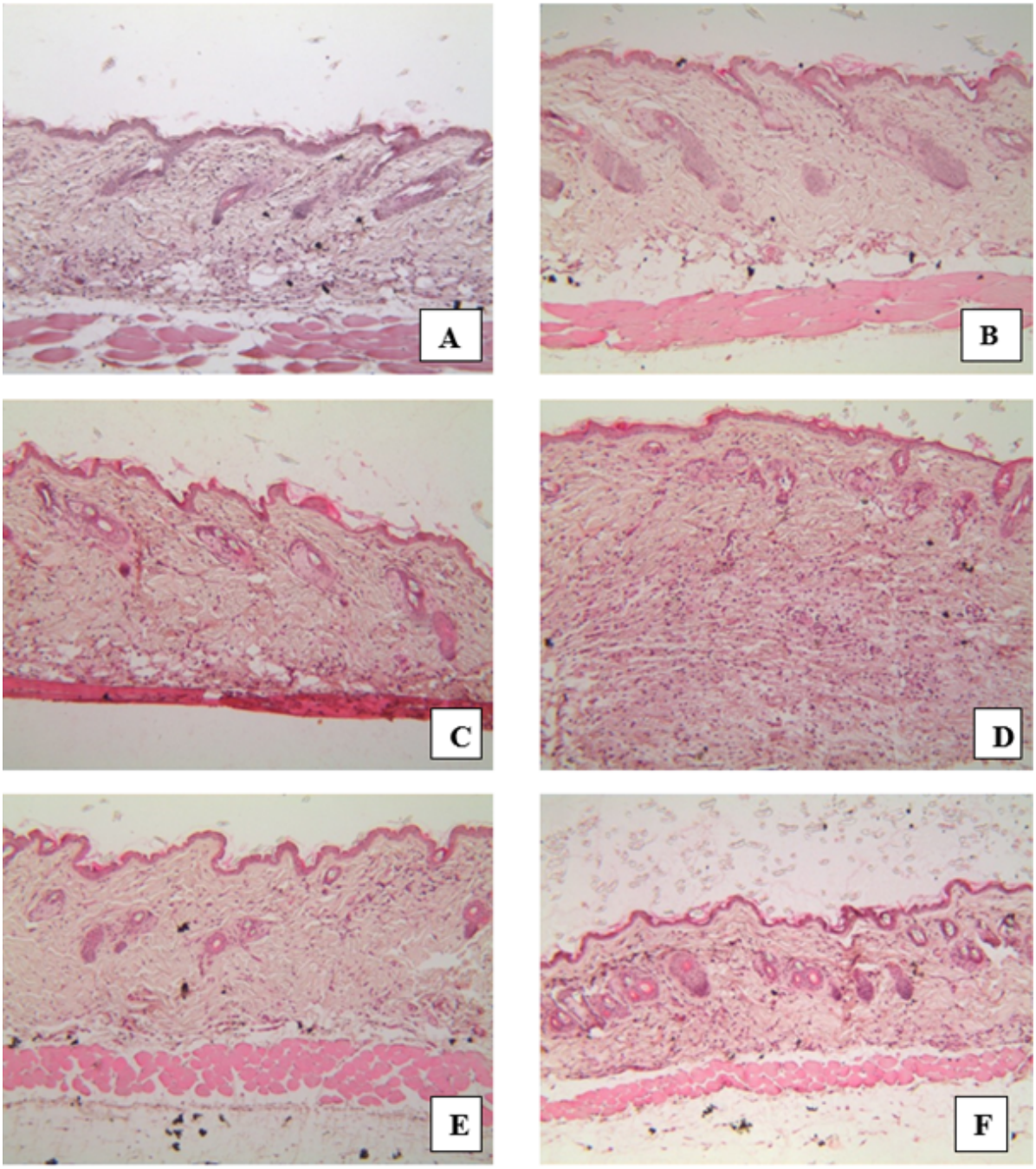


Figure 1. Morphological changes of ageing mice skin induced by D-gal in deferring -organism-ageing experiment. Hematoxylin and eosinstained skin of treated mice. (a) control group; (b) model group; (c) VC group ; and PTSE groups.

Polyphenolic compounds such as phenolic acid, flavonoids and tannins are commonly found in seed extract of *P. tectorius*. Moreover, phenols and polyphenolic compounds are widely found in pharmaceutical products derived from plant sources, and they have shown to possess significant antioxidant activity as well.[24] The studies have shown that these extracts are significant source of natural antioxidants which might be helpful in preventing the progress of oxidative damage. Another study evaluated the antioxidant potential of extract from *Pandanus tectorius* and found the highest content of phenolics and flavonoids in it.[12] Phenolic compounds are also effective hydrogen donors, which make them good antioxidants.[25] Thus, the therapeutic properties of PTSE may possibly be attributed to the phenolic compound present in it.

## Conclusion

The results of the our deferring skin-ageing experiment showed similar skin caring activity of both VC and PTSE. Both of them were found to effectively enhance SOD activity, maintain collagen density and reduce the MDA content in the

ageing mice skin. Our data suggested that PTSE delays skin ageing via strengthening the antioxidant capacity to some extent. Furthermore, its effect was not less than that of the well-known VC. The therapeutic properties of PTSE may possibly be attributed to the phenolic compound present in it. These results seem to be implying that *Pandanus tectorius'* seed extract has great potential of developing anti-ageing medicines and skin care products.

## Conflict of Interests

The authors declare that there is no conflict of interest.

## Acknowledgment

The present work was financially supported by the Directorate of Research and Community Services, Universitas Nasional and the authors are grateful to the Faculty of Biology, Universitas Nasional, Jakarta, Indonesia for providing space in the animal house.

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