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Beetroot (Beta vulgaris) Extract Effectiveness as an Anti-Aging, Nephroprotective, and Anti-Hypercholesterolemic Agent in D-Galactose Induced Wistar Rats

Jesslyn, Linda Chiuman, Chrismis Novalinda Ginting, Sulaiman Delrizal, Sahna Ferdinand

Department of Biomedical Science, Universitas Prima, Indonesia

ABSTRACT

Objective: to determine the effectiveness of beet root extract as an anti-aging, nephroprotector, and anti-hypercholesterolemia in Wistar rats induced with D-galactose to maximize the use of beetroot as a health supplement.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Laboratory of Faculty of Medicine, Universitas Prima, Indonesia, and Pharmacy Laboratory, Universitas Sumatera Utara, Indonesia, from Jun 2023 to Aug 2023.

Methodology: The study sample consisted of 25 male Wistar rats aged six to eight weeks that were obtained from the Pharmacy Laboratory and had gone through the inclusion and exclusion criteria. The sample was then induced by D-galactose and given an intervention using beetroot extract and vitamin C.

Results: Beetroot extract at a dose of 500 mg/kg body weight showed more significant results as anti-aging, nephroprotector, and anti-hypercholesterolemic compared to 250 mg/kg body weight. Mean value of Group C for anti-aging was 136.26±15.16 (keratinocyte cells) and 35.93±10.64 (fibroblast cells), which was slightly lower than Group D, 136.86±4.29 (keratinocyte cells) and 43.80±5.80 (fibroblast cells). Ureum mean value of Group C for nephroprotector was 79.00±3.16, which was significantly lower than Group D (127.20±6.26) and Group E (105.00±4.63), but creatinine in Group E was the lowest with a mean value of 105.00±4.63 compared to Group C (0.83±0.08) and Group D (0.74±0.07). Group E had the lowest mean value, 105.00±4.63, for total cholesterol compared to Group C (165.00±16.12) and Group D (127.20±6.26).

Conclusion: This experimental study proved the positive effects of beet root extract as anti-aging, nephroprotective, and antihypercholesterolemic on the health of Wistar rats.

Keywords: Beetroot Extract, Organoprotector, Skin Aging.

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INTRODUCTION

Aldohexose, a reduction sugar, is a naturally occurring compound in the body and in a variety of foods, including milk, butter, cheese, yogurt, honey, beetroot, plums, cherries, figs, and celery.[1] Under the catalysis of the enzyme galactose oxidase, galactose can be converted to aldose and hydroperoxide at high concentrations, resulting in the production of reactive oxygen species (ROS).[2] Further increases in ROS can result in oxidative stress, inflammation, mitochondrial dysfunction, and cell apoptosis.[3] The aldose reductase enzyme will help convert this galactose into galactitol. This galactitol was unable to be digested by the body, so it accumulated. Changes in osmotic pressure would cause cells to expand. In addition, damage to cells can also occur.[4]

Ascorbic acid, carotenoids, phenols, flavonoids, and betalains were some of the phytochemicals found in beetroot that had strong antioxidant activity.[5] Beetroot contains antioxidants called polyphenols, flavonoids, and betalains that contribute to preventing dyslipidemia.[6] Through the inhibition of ROS (reactive oxygen species), the antioxidant content in beetroot extract activity may contribute to its hepatoprotective and nephroprotective effects.[7] Due to the many benefits of beetroot, researchers are very interested in conducting research to determine the effects of beetroot as an anti-aging, nephroprotective, and anti-hypercholesterolemia effect compared to vitamin C on d-galactose-induced rats.

METHODOLOGY

This study was an experimental study with a true experimental design using a posttest-only Control Group design that was intended to discover beet root extract as an anti-aging, nephroprotector, and anti-hypercholesterolemic compared to vitamin C. The samples were partitioned into five Groups, each Group consisting of a minimum of 5 samples, using

Correspondence: Dr Jesslyn, Department of Biomedical Science, Universitas Prima, Indonesia.

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Federer's formula, which was (t-1)(n-1) >15 (t: number of intervention/Group; n: total sample of each Group).

Inclusion Criteria: Male Wistar rats aged six to eight weeks, body weight 150-200 grams, active, and had never been used in research were included.

Exclusion Criteria: Mice with any obvious injury, anatomical abnormalities and disease were excluded.

The study sample consisted of 35 total samples, which had gone through inclusion criteria In this research, two samples of each Group died. Subsequently, the samples were put in cages measuring 25x25x25 cm and provided with standard food and drink for a duration of 1 week. The samples were partitioned into five Groups, such as: Normal Control Group (A): Group that was not administered D-galactose and did not receive any beet root extract. This Group received standard food and water for a period of 10 weeks, Negative Control Group (B): Group that was administered D-galactose at a dosage of 500 mg/kg BW for 6 weeks without any beet root extract. This Group received standard food and water for a period of 10 weeks. Experimental Group I (C): Group that receives a dosage of 500 mg/kg BW of D-galactose for 6 weeks, followed by beet root extract at a dosage of 250 mg/kg BW for 4 weeks. They were provided with standard food and water for a total of 10 weeks. Experimental Group II (D): Group that received a dosage of 500 mg/kg BW of D-galactose for 6 weeks, followed by beet root extract at a dosage of 500 mg/kg BW for 4 weeks. They were provided with standard food and water for a total of 10 weeks. Positive Control Group (E): Group that receives a dosage of 500 mg/kg BW of D-galactose for 6 weeks, followed by Vitamin C at a dosage of 28 mg/kg BW for 4 weeks. Throughout the entire 10-week period, the Group was provided with standard food and water.

10 kg of Beet root were washed and sliced with a thickness of about 1 mm to form chips. Then the beet roots are dried for 6x24 hours in the oven at 40 °C until they become simplicia. The simplicia were obtained, then blended and sieved to obtain a fine powder.

The extraction process begins by soaking 25 grams of beet root simplicia into 50 mL of 96% ethanol solvent for 3x24 hours and stirring periodically, then filtered using filter paper. The distillate was then concentrated using a rotary vacuum evaporator. After obtaining 10ml of concentrated extract, the concentrated extract was left for several days in the refrigerator at a temperature of 4-10 °C, covered with aluminium foil. The extract beet root solution is prepared by dissolving 10mg of ethanol extract in a small amount of 1% DMSO solution, and then adding sodium phosphate buffer solution (pH 7.5) until a final volume of 10mL is reached. The resulting solution has a concentration of 2.5mg/mL of extract from beet root.

There were several parameters that were examined in this study, as for anti-aging, which using skin histopathology had several parameters such as thickness of stratum corneum, quantity of keratinocyte cells, quantity of fibroblast cells, and collagen density.

Statistical Package for Social Sciences (SPSS) version 25.0 was used for the data analysis. As for nephroprotector, which uses kidney histopathology such as tubular, endothelium, glomerulus, and tubulointerstitial, and laboratory tests such as ureum and creatinine. Moreover, as for anti-hyper-cholesterolemia, using laboratory test total cholesterol results. This study used the Kruskal-Wallis test for non-parametric variables, such as stratum corneum thickness and collagen density (as anti-aging); tubulus, endothelium, glomerulus, and tubulointerstitial (as nephroprotective), and the Way ANOVA test for parametric variables, such as quantity of keratinocyte cells and quantity of fibroblast cells (as anti-aging); Ureum and Creatinine (as nephroprotective), and cholesterol total (as anti-cholesterolemic). The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

A total of 35 samples of Wistar rats were enrolled in this study, out of which 35(100%) were male Wistar rats, with a mean weight of 174.74±13.79 grams, and partitioned into 5 Groups, each Group needed a minimum of 5 samples according to Federer's formula. This study consists of 7 samples in each Group, and 2 samples of each Group died during the intervention. Median value of Group C and Group D had similar results compared to Group E, as shown in Table-I.

Table-I: Median Values of Anti-aging and Nephroprotector (n=25)

Parameters	Study Groups					*p*-value
	Group A (n=5)	Group B (n=5)	Group C (n=5)	Group D (n=5)	Group E (n=5)	
Stratum corneum thickness*	2(1)	2(0)	1(1)	1(0)	1(0)	0.006
Collagen density*	3(1)	3(0)	4(1)	4(1)	4(0)	0.020
Tubulus**	0(0)	3(1)	2(1)	1(0)	1(1)	<0.001
Endothelium**	0(0)	2(1)	2(2)	2(0)	1(1)	0.001
Glomerulus**	0(0)	2(1)	1(1)	1(2)	0(0)	0.001
Tubulo-interstitial**	0(0)	2(1)	1(2)	1(0)	0(1)	0.006

Mean value of Group C for anti-aging was 136.26±15.16 (keratinocyte cells) and 35.93±10.64

(fibroblast cells), which was slightly lower than Group D, 136.86±4.29 (keratinocyte cells) and 43.80±5.80 (fibroblast cells). Ureum mean value of Group C for nephroprotector was 79.00±3.16, which was significantly lower than Group D (127.20±6.26) and Group E (105.00±4.63), but creatinine in Group E was the lowest with a mean value of 105.00±4.63 compared to Group C (0.83±0.08) and Group D (0.74±0.07). Group E had the lowest mean value, 105.00±4.63 for total cholesterol, compared to Group C (165.00±16.12) and Group D (127.20±6.26), as shown in Table-II. The Inter-Group comparison is shown in Table-III. Skin Histopathology and Nephron Histopathology of study Groups are shown in the Figure-1 and Figure-2, respectively.

Table-II: Mean Values of Anti-aging, Nephroprotector and Total Cholesterol (n=25)

Parameters	Study Groups					*p*-value
	Group A (n=5)	Group B (n=5)	Group C (n=5)	Group D (n=5)	Group E (n=5)	
Quantity of keratinocyte cells	73.06±2.55	64.33±12.39	136.26±15.16	136.86±4.29	123.39±18.09	<0.001
Quantity of fibroblast cells	13.66±1.74	13.86±3.00	35.93±10.64	43.80±5.80	51.20±4.59	<0.001
Ureum	43.20±2.58	102.60±3.36	79.00±3.16	127.20±6.26	105.00±4.63	<0.001
Creatinine	0.55±0.08	1.05±0.14	0.83±0.08	0.74±0.07	0.62±0.07	<0.001
Total Cholesterol	46.20±5.26	208.60±18.95	165.00±16.12	127.20±6.26	105.00±4.63	<0.001

Table-III: Inter-Group Comparison; (Post Hoc analysis) of Anti-aging, Nephroprotector, and Total Cholesterol (n=25)

Group Comparison	Post Hoc Test	Group B vs Group C	Group B vs Group D	Group B vs Group E	Group C vs Group D	Group C vs Group E	Group D vs Group E
Quantity of keratinocyte cells	Games-Howell	<0.001	<0.001	0.003	1.000	0.742	0.550
Quantity of fibroblasr cells	Bonferroni	<0.001	<0.001	<0.001	0.514	0.007	0.656
Ureum	Bonferroni	<0.001	<0.001	1.000	<0.001	<0.001	<0.001
Creatinin	Bonferroni	0.020	<0.001	<0.001	1.000	0.020	0.618
Total Cholesterol	Games-Howell	0.028	0.002	0.001	0.021	0.004	0.002

DISCUSSION

Aldohexose, a reduction sugar, is a naturally occurring compound found in the body and various foods. These include milk, butter, cheese, yogurt, honey, beetroot, plums, cherries, figs, and celery.[8] Under the catalysis of the enzyme galactose oxidase, galactose can convert to aldose and hydroperoxide at high concentrations. This results in the production of reactive oxygen species (ROS).[2] Continued elevation of ROS levels can cause oxidative stress, inflammation, impaired mitochondrial function, and cell death.[9]

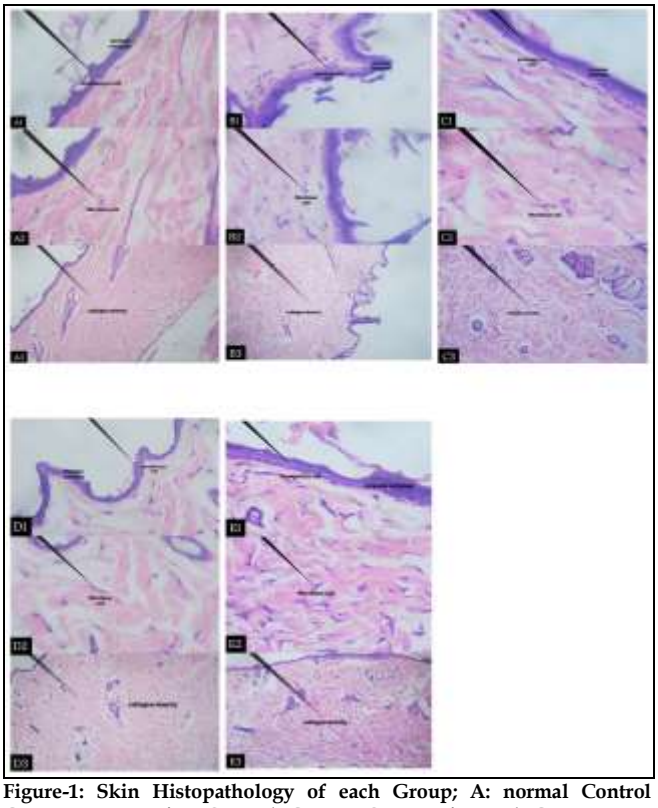

Figure-1: Skin Histopathology of each Group; A: normal Control Group, B: Negative Control Group; C: Experimental Group I; D: Experimental Group II; E: Positive Control Group; 1: Stratum Corneum and Keratinocyte Cell; 2: Fibroblast Cell; 3: Collagen Density

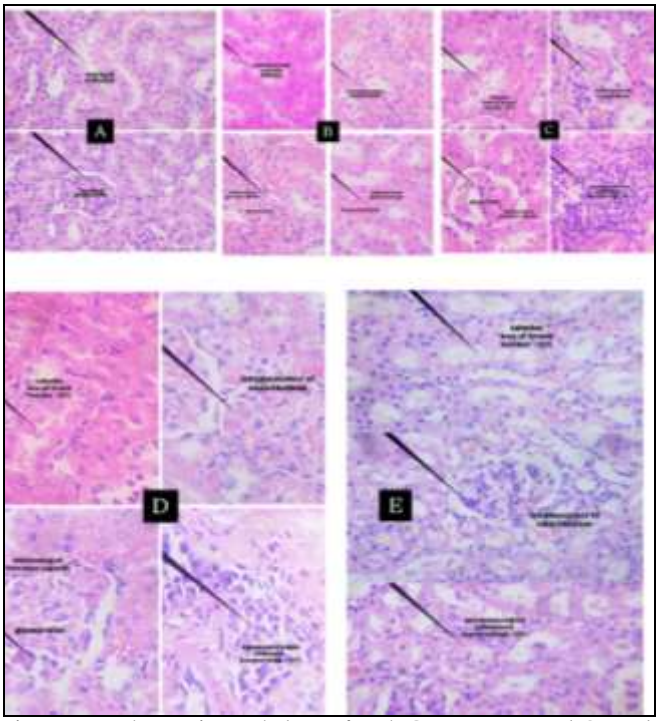

Figure-2: Nephron Histopathology of each Group; A: normal Control Group; B: negative Control Group; C: experimental Group I; D: experimental Group II; E: positive Control Group

Beetroot (Beta vulgaris) contains bioactive components, including betanin, antioxidant substances, and phenolic compounds. Additionally, it provides a substantial amount of dietary fiber, as well as an array of minerals such as potassium, sodium, iron, copper, magnesium, calcium, phosphorus, and zinc, and vitamins such as retinol, ascorbic acid, and B-complex.[9,10] In Praveen *et al.,* research study, they obtain the presence of saponin, quinone, flavonoid, phenols, couarin, steroid, anthocyanin, and betacyanin, and the absence of tannin and glucoside.[11] Phenolic compounds found in some plants have a remarkable ability to scavenge free radicals, thus effectively slowing down the aging process.[12]

Beetroot (Beta vulgaris) possesses elevated concentrations of antioxidants that are efficacious in combating aging.[13] Beetroot comprises anti-free radical vitamins such as vitamin A, vitamin C, folic acid, and vitamin B-9. Vitamin A can counteract age-related skin thinning, rejuvenate collagen production, and rectify uneven skin pigmentation. Vitamin C aids in rectifying uneven skin pigmentation, synthesizes skin collagen, and efficiently combats free radicals to mitigate skin damage. Research studies have demonstrated that elevated amounts of folic acid and antioxidants can effectively decrease the depth and severity of skin wrinkles by as much as 60%.[14,15] In these research studies, all anti-aging parameters had a *p*-value <0.05. This means beetroot extract had a significant effect as an anti-aging agent. Therefore, beetroot extract can be used as an anti-aging agent.

In research study by Amelia *et al.,* and Chen *et al.,* bioactive compounds were obtained: nitrate, nitrite, saponins, organic acids, phenolic compounds, and betalains. Betanin, from the bioxanthin group, is the most prevalent antioxidant in beetroot (Beta vulgaris). This group is a primary category of betalain. Betanin's potent antioxidant capabilities come from a phenolic hydroxy group and a benzene group with unsaturated bonds. Identifying betanin in beetroot (Beta vulgaris) helps safeguard vital organs by counteracting the detrimental effects of oxidative stress in several chronic ailments.[16,17] In these research studies, all nephron parameters had a *p*-value <0.05. This means beetroot extract had a significant effect as a nephroprotective agent. Therefore, beetroot extract can be used as a nephroprotective agent.

Beetroot (Beta vulgaris) has two primary betalain pigments: red betanin and yellow vulgaxanthin.[18,19] A beetroot formulation with solid betalain efficiently preserved blood lipid profile. It elevated the HDL to LDL cholesterol ratio and reduced oxidized LDL.[20]

In a separate trial, LDL cholesterol levels were reduced in 30 healthy individuals who received 250 mL of beetroot (Beta vulgaris) juice with 300 grams of glucose. The beetroot juice further reduced overall lipid levels.[21] Another study found that a food supplement high in betalains effectively reduced cholesterol, triglyceride, and LDL levels in 48 male patients.[22] In these research studies, the total cholesterol parameter had a *p*-value <0.05. This means beetroot extract had a significant effect as an anti-hypercholesterolemia agent. Therefore, beetroot extract can be used as an anti-hypercholesterolemia agent.

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CONCLUSION

This experimental study proved that beetroot extract has positive anti-aging, nephroprotective, and antihypercholesterolemic effects on the health of Wistar rats. A dose of 500 mg/kg body weight was more effective than 250 mg/kg body weight. Using beetroot ethanol extract to treat skin aging increases the number of keratinocyte and fibroblast cells. It also decreases stratum corneum thickness and increases collagen density. The extract reduces levels of ureum, creatinine, and inflammation in nephron cells, as observed in nephron histopathology. It has also been found to decrease total cholesterol levels in rats stimulated with D-galactose. As an antioxidant, it yields outcomes nearly identical to vitamin C.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

J & LC: Data acquisition, data analysis, critical review, approval of the final version to be published.

CNG & SD: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SF: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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