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THE EFFECT OF RED DRAGON FRUIT PEEL EXTRACT (*HYLOCEREUS POLYRHIZUS*) ON COLLAGEN DEPOSITION IN THE KIDNEYS OF MENOPAUSAL MODEL RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

Background: The estrogen hormone possesses nephroprotective properties during menopause that can influence kidney function. This increases collagen deposition in the kidneys. Estrogen deficiency during menopause can be replaced by phytoestrogens. The skin of red dragon fruit (*Hylocereus polyrhizus*) contains numerous anthocyanin contents, which have been proven to exhibit activities as phytoestrogens.
Objective: To determine whether there are changes in collagen deposition in the kidneys of menopausal rat models with the administration of *Hylocereus polyrhizus* peel extract.
Methods: This research utilized a randomized post-test control group design with three sample groups that had undergone ovariectomy. The three groups consist a control group without intervention (P0), an intervention group with red dragon fruit peel extract at a dose of 60mg/200gBW (P1), and an intervention group with red dragon fruit peel extract at a dose of 90mg/200gBW (P2).
Results: The mean percentage of collagen expression in menopausal rat kidneys in groups P0, P1, and P2 were 0.876 ± 0.433, 0.189 ± 0.102, and 0.162 ± 0.108, respectively. The Kruskal-Wallis test revealed a significant difference with a p-value of 0.000. Post-Hoc Mann-Whitney U test found a significant difference between groups P0 and P1 (p = 0.000), as well as groups P0 and P2 (p = 0.000). However, there was no significant difference between P1 and P2 (p = 0.436).
Conclusion: The extract of Hylocereus polyrhizus peel has an effect on the collagen deposition in the kidneys of menopausal rat models. The lowest level of collagen deposition in the kidneys was observed in the intervention group with a dose of 90mg/200gBW.

Keywords : Hylocereus polyrhizus, collagen, kidney, menopause

INTRODUCTION

Menopause is the permanent cessation of the menstrual cycle and the onset of estrogen deficiency or hypoestrogenism in the body[1]. Hypoestrogenic conditions lead to significant physiological changes in vital organs of menopausal women, including the kidneys[2]. Kidney collagen deposition in hypoestrogenic conditions is an initial stage in the development of physiological dysfunction in women's kidneys, especially in menopausal women with hypoestrogenism[3]. This is associated with the nephroprotective effects of estrogen[4]. The drastic decline in estrogen levels in menopausal women is typically addressed through hormone replacement therapy (HRT)[4]. However, the effectiveness of HRT has been shown to be disproportionate to the risks of breast cancer, pulmonary embolism, and dementia.[2] Alternative natural, risk-free, and affordable estrogen replacement therapy becomes the solution to the issues associated with detrimental effects of HRT on menopausal women.[5]

Phytoestrogens are non-steroidal substances in plants that have estrogenic effects.[6] One type of phytoestrogen, isoflavones, has a structure similar to estradiol estrogen and can affinity-bind with estrogen receptors.[7] The anthocyanin content in red dragon fruit has a structural similarity to isoflavones, also known as flavonoids, and can act as a substitute for estrogen in menopausal women. This substitution may help reduce menopausal issues, particularly preventing collagen deposition as an early stage in kidney-related problems during menopause, including kidney fibrosis and glomerulosclerosis[3,5,8].

METHODS

This research activity employed a randomized post-test only control group design on in vivo animal models with approval from the research ethics committee of the Faculty of Medicine, Udayana University. Female rats weighing 180-220 grams and aged 3 to 4 months, which had undergone bilateral ovariectomy and met inclusion and exclusion criteria, were used in this study. The sample size was determined using the Federer formula, resulting in 9 rats per group, divided into three groups: Group P0 (control), Group P1, and Group P2. To account for potential rat mortality in each group, one extra rat was added, bringing the total number of research samples to 30 rats, randomized evenly

across the groups. Group P0 served as the control group receiving 1 ml/day of normal saline, while Group P1 received red dragon fruit peel extract at a dose of 60mg/200gBW/day, and Group P2 received a higher dose of 90mg/200gBW/day. Each group received treatment according to its respective category over a predetermined period of 30 days.

The red dragon fruit peel extract was prepared from the processing of red dragon fruit (*Hylocereus polyrhizus*) peel. The red dragon fruit used in the study was obtained from a supermarket in Denpasar, and the selection criteria included ripe and red-colored fruits. Subsequently, the fruit peel and pulp were separated by peeling and washing with clean running water. The peel was then sliced, dried, roasted, and ground sequentially using a blender. After grinding into coarse powder, the next step involved sieving to obtain fine powder. The fine powder was then macerated with a 96% ethanol solution at a ratio of 1:7 for three days with stirring twice a day. After maceration, the extract was separated using filter paper, and the solvent was evaporated to obtain a concentrated red dragon fruit peel extract.

Ovariectomy in Wistar rats was performed using the modified method of Ingle DJ and Grith JQ. First, the rats were anesthetized with ketamine at a dose of 40 mg/kgBW via intramuscular injection. Subsequently, the abdominal area was shaved, prepared, sterilized, and covered with a sterile drape. A transabdominal incision above the rat's uterus was made, layer by layer, measuring 1.5 to 2 cm until penetrating the peritoneal wall. The uterus was then located, and the search continued for the left uterus-oviduct-ovary complex. The oviduct and ovary were cleaned, tied, and removed. The same procedure was carried out on the right side. Finally, the incision was closed layer by layer using stitches. After ovariectomy, rats received post-operation therapy with gentamicin injections at a dose of 60 to 80 mg/kgBW/day for three consecutive days. This process was performed on all 30 rats used in the study.

Histological preparations of rat kidneys were stained with Picro-Sirius Red, fixed, and observed under an Olympus CX41 microscope at 40x magnification. Three fields of view were photographed using an Optilab Pro camera. Photo processing was conducted using ImageJ software to calculate the percentage of red-colored collagen pixels due to the previous staining. The percentage data were collected in an Excel spreadsheet and analyzed.

The percentage of collagen pixel data for each sample was analyzed using IBM SPSS Statistics 23.0. Data analysis included descriptive and group mean tests, normality testing using the Shapiro-Wilk method, homogeneity testing with Levene's Statistic, and comparative testing. Due to non-normally distributed and heterogenic data, the Kruskal-Wallis non-parametric test was conducted, followed by Post-Hoc testing using the Mann-Whitney method to determine comparisons.

RESULTS

After observations using a microscope at 40x magnification, areas of red-colored collagen were found in the kidneys in several parts, such as the interstitium, around the glomerulus, and around the arteries. In the P0 group or the control group (**Figure 1**), the observed collagen was identified as type 1 collagen deposition that filled some interstitial spaces, around the glomerulus, and around the arteries. The evaluated collagen appeared denser around the arteries, while in the interstitium and glomerulus, it was not denser than around the arteries.

In the P1 and P2 groups, or the test groups with respective doses of 60mg/200gBW (**Figure 2**) and 90mg/200gBW (**Figure 3**), it can be observed that the collagen area is not as extensive as in the control group, but both groups showed insignificant differences. Similar to the control group, in the P1 and P2 groups, the evaluated collagen formed appeared denser around the arteries, while in the interstitium and glomerulus, it was not denser than around the arteries.

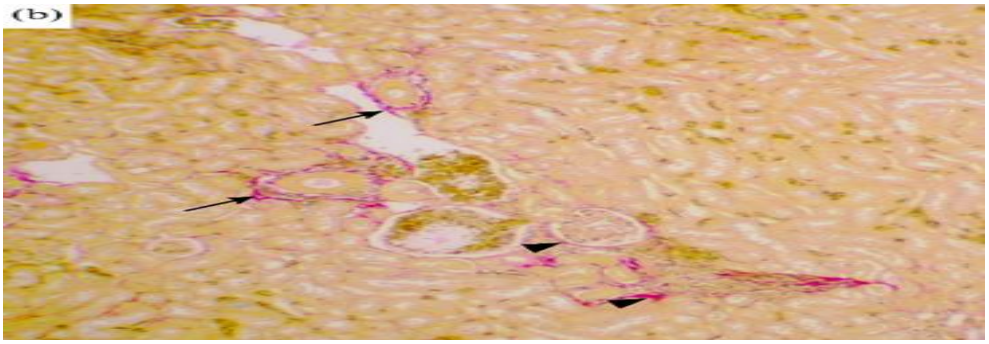

Figure 1. Histological Image of Kidneys at 40x Magnification in Group P0. Collagen formation was found around the arteries (arrowhead) and in the interstitium and around the glomerulus (arrow).

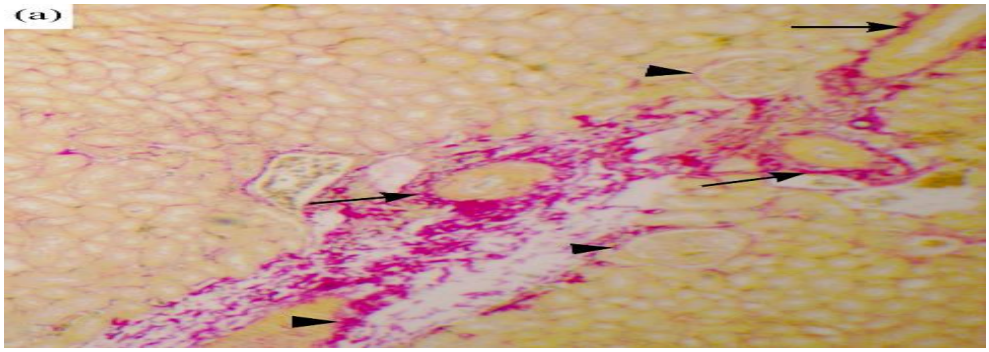

Figure 2. Histological Image of Kidneys at 40x Magnification in Group P1. Collagen formation was found around the arteries (arrowhead) and in the interstitium and around the glomerulus (arrow).

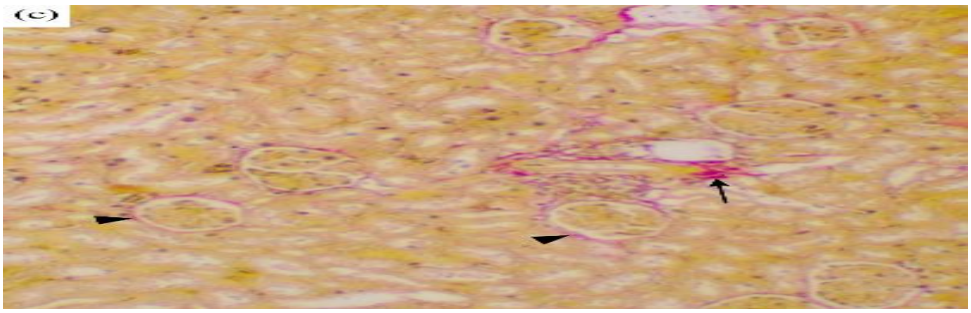

Figure 3. Histological Image of Kidneys at 40x Magnification in Group P2. Collagen formation was found around the arteries (arrowhead) and in the interstitium and around the glomerulus (arrow).

Based on the results of the descriptive analysis of each subject group (**Table 1**), the highest average percentage of kidney collagen area was found in the control group or P0. Following that, the average percentage of kidney collagen area was observed in the P1 group with the intervention dose of red dragon fruit peel extract at 60mg/kgBW/day. In the last position, the lowest average percentage of kidney collagen area was obtained in the P2 group with the intervention dose of red dragon fruit peel extract at 90mg/kgBW/day.

Table 1. The average collagen area of the subject groups

Group	Mean (%)	N	Std. (%)
P0	0,8763	10	0,43300
P1	0,1897	10	0,10270
P2	0,1623	10	0,10847

Next, the normality test of the data was conducted using the Shapiro-Wilk method. The results of the normality test showed that there was non-normally distributed data, specifically in the P2 group ($p < 0.05$). Meanwhile, the P0 and P1 groups had normal distributions ($p > 0.05$). Subsequently, the homogeneity test of the data was conducted using the Levene Statistic method. The results of the homogeneity test revealed that the data were heterogeneous ($p = 0.000$). A non-parametric test was performed using the Kruskal-Wallis method. The results of the non-parametric test indicated a significant difference in the mean collagen area among the three subject groups ($p = 0.000$).

Post-Hoc tests using the Mann-Whitney U method were carried out to identify significant differences between specific pairs of treatments statistically. The results of this Post-Hoc test showed significant differences between the P0 and P1 groups, and between the P0 and P2 groups, while there were no significant differences between the P1 and P2 groups (**Table 2**).

Table 2. Results of the Post-Hoc test using the Mann-Whitney U method for the three subject groups

Groups	Mann-Whitney Test	The difference in mean ranks	P-value
P0 vs P1	1,000	9,8	0,000
P0 vs P2	2,000	9,6	0,000
P1 vs P2	39,000	2,2	0,436

DISCUSSION

A study has been conducted on the influence of red dragon fruit peel extract on collagen deposition in the kidneys of menopausal rats, with the following results: in both groups given red dragon fruit peel extract doses, collagen formation was lower than in the control group given a placebo. Artificial menopause conditions were created by performing bilateral ovariectomy on all samples, resulting in hypoestrogenic conditions, or estrogen hormone deficiency, similar to menopausal women. In menopausal women, hypoestrogenic conditions are closely related to structural changes in the kidneys, especially excessive collagen deposition leading to kidney fibrosis and glomerulosclerosis[3].

The formation of kidney fibrosis and glomerulosclerosis under hypoestrogenic conditions is due to the loss of the nephroprotective effect of estrogen. Therefore, structural changes and a decline in kidney function are associated with gender differences. This is evidenced by a gradual decrease in glomerular filtration rate in menopausal women aged 50 and above[9]. Similar patterns are observed in menopausal women, who have the highest prevalence of end-stage kidney failure[4].Previous research has stated that the formation of glomerulosclerosis in post-menopausal female rats can be prevented with estrogen supplementation[4]. In clinical cases, hormone replacement therapy (HRT) is administered to menopausal women to reduce specific symptoms and prevent complications, including kidney failure[5]. However, the effectiveness of HRT is not proportional to its risks, leading to the need for natural substances with the potential to replace estrogen[5].

Phytoestrogens can be found in everyday foods and possess estrogenic activity due to their structural similarity to 17β-estradiol[6]. In previous research, one type of phytoestrogen, isoflavone, such as genistein found in soybeans, demonstrated estrogenic effects[10]. Red dragon fruit peel contains anthocyanins with a structure similar to genistein, capable of substituting for estrogen in menopausal women by occupying estrogen receptors[11].The research results on the red dragon fruit peel extract's effect on collagen formation showed that the percentage of collagen area in the treatment groups with doses of 60mg/200gBW (P1) and 90mg/200gBW (P2) was significantly lower than in the control group. In other words, the red dragon fruit peel extract can suppress collagen formation in the kidneys. This aligns with a study by Yulista et al. in 2021, which found that ethanol extract of red dragon fruit peel at doses of 60mg/200gBW and 90mg/200gBW could prevent significant structural changes in the kidneys as an initiation of chronic kidney disease[12]. The effectiveness of red dragon fruit peel extract is consistent with a study by Hernowo et al. in 2023, which found that *Hylocereus polyrhizus* extract influenced the reduction of hepatic steatosis in menopausal rats[15]. Supported by previous research, the anthocyanin content in red dragon fruit peel can provide estrogenic activity due to its structural similarity to genistein[13]. This is evidenced by a study by Farahmand et al. in 2021, showing that genistein can bind to estrogen receptor-β and suppress the formation of collagen type I by increasing matrix metalloproteinase-9, preventing the formation of extracellular matrix components[3]. In other words, the nephroprotective effect in preventing collagen formation in the kidneys can be provided by red dragon fruit peel extract with its anthocyanin content.

Most of the collagen density was found in the interstitium and around the glomerulus in the control group, marked by red-colored areas. This is theorized to occur due to the synthesis of type I collagen from the accumulation of extracellular matrix by mesangial cells in the glomerulus[3]. Further findings, predominantly around the arteries, are likely related to hypertension associated with estrogen deficiency. This occurs due to a decrease in the elasticity of arterial muscles in the kidneys due to hypertension suppressing ERα activation[14].

After administering a specific dose of red dragon fruit peel extract, the formed kidney collagen in the P1 and P2 groups was not as much as in the control group. In other words, red dragon fruit peel extract has a significant impact on reducing collagen deposition. This is supported by the Kruskal-Wallis test results, indicating a significant difference among the three subject groups. However, the Mann-Whitney test revealed that the control group differed significantly from P1 and P2, while the difference between P1 and P2 was not significant. The dosage difference given to the P1 and P2 groups demonstrates that even the smallest dose can make a significant difference. However, further research with a longer duration and better dose calculations is needed to determine the effectiveness of red dragon fruit peel in addressing menopausal symptoms, particularly in the kidneys.

CONCLUSION

Based on the results of this research, it can be concluded that the ethanol extract of red dragon fruit peel (*Hylocereus polyrhizus*) given to the menopausal rat model has an effect on kidney collagen deposition, resulting in a lesser amount of collagen formation in the treatment groups compared to the control group.

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