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# Biochemical and Immunohistochemical Study of the Effect of Rhubarb (*Rheum ribes*) against the Oxidative Stress on the Brain and Pancreas in an Animal Model

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**ABSTRACT**

The study was designed to investigate the effect of Rhubarb (*Rheum ribes*) on the brain and pancreas of male rats affected by aging. A total of 24 white albino rats aged three month old were used in this experiment. The samples were divided into four groups, each containing six rats. The rats in the first group were administrated normal saline and set as a control group. Those in the second group were injected with 500 mg/kg of D-galactose (D-gal) for 40 days. Animals in the third group were administrated with *Rheum ribes*. The fourth group was subjected to both D-gal and *R. ribes*. At the end of the study, blood serum, pancreas and brain samples were collected. The obtained results indicated a considerable increase in the glucose, melanoaldihide and beta-amyloid levels (P≤0.01). On the other hand, glutathione decreased significantly in rats in the D-gal group (P≤0.01), compared to other groups. The fourth group revealed a significant rise in glucose, melanoaldihide and beta-amyloid levels (P≤0.01) and a decrease in glutathione levels when compared to the control and *R. ribes* groups. Immunohistochemically, the study showed that D-gal group was strongly positive to the stain, while the combination group indicated a weak positive to the immunohistochemical stain. Control and *R. ribes* group revealed a negative result when staining with immunohistochemical stain. The results suggest that *R. ribes* extract has some anti-hypoglycemic effects. However, further studies are needed to confirm these findings.

**Key words:** *Rheum ribes,* oxidative stress, immunohistochemical assays

## INTRODUCTION

Aging is a biological process that causes a malfunction in several organs of the body throughout an individual's life, and it differs from person to person. Individuals' pathological and psychological changes lead to aging (Hägg and Jylhävä, 2021). Chronic illnesses, such as diabetes, alzheimer's and cardiovascular ailments are all exacerbated by aging. It proves that aging causes a beta-cell malfunction in the pancreas. Insulin resistance develops with aging as a result of increased free radical generation and oxidative stress. D-galactose (D-gal) can cause aging in rats and mice and increase oxidative stress by making free radicals. Excessive release of free radicals and damage to human organs caused by oxidative stress lead to an imbalance between antioxidant defense and free radical production (Mahdi and Hussain Al-Aameli, 2021). The root of *R. ribes* (Rhubarb) is one of the oldest and most well-known Chinese medicinal herbs (Pang *et al.*, 2018). In alloxan-induced diabetic mice, a decoction extract of *R. ribes* roots has been shown to have a significant hypoglycemic effect and blood sugar-reducing action (Husseini *et al.*, 2017). Moreover, one of the most important crude medications in West Asiatic regions comes from *R. ribes*. The plant entailes plentiful amounts of vitamins A, B and C. Traditional Syrian rhubarb root (Rhizoma Rhei ribi) treatments include diabetes, hemorrhoids, ulcers and diarrhea. In Bitlis, Turkey, the herb is also used as a digestive and appetizer. Anemia, anorexia, weakness, anxiety, sadness and diabetes are all treated with this traditional herbal remedy stem and root dry plant (Kasapoglu *et al.* 2020). *R. ribes* has long been used as a sedative and mood enhancer in Iran (Andarkhor *et al.*, 2019).

## MATERIALS AND METHODS

Plant sampling preparation was the first stage in researching the therapeutic properties of plants since it preserves the biomolecules in the herbs prior to collection. To extract pieces such as rhizomes, fresh or leaf powder of *R.*

*ribes* can be used. Other pre-treatments of bioactive components of this herb, such as drying and grinding, affect the retention of phytochemicals in the final extracts (Kewlani *et al.,* 2022).

Primary processing refered to the immediate post-harvest treatments given to herbs harvested through cultivation, wildcrafting, or field collecting in order to remove residues of unwanted plant parts and other impurities. This process included sorting (garbling), washing and aerial drying powdered samples. Accordingly, a more homogenized sample with smaller-sized particles resulted in increased surface contact with extractants. For excellent extraction, it was necessary to contact the target analyses, and particles less than 0.5 mm were ideal for solubilization.

The maceration extraction process was used to extract bioactive components from plants. Plant materials (coarse or powdered) were soaked in a sterile container with a liquid, then it was left still at room temperature for at least three days with constant stirring, followed by compressing or separating and filtration. Heat was transferred by convection and conduction in traditional methods, and the extractor was chosen based on the substance to be extracted. Infusion and decoction were similar to maceration in that they were immersed in cold water.

Rats were divided into four groups (six rats per group), including one control group and three treatments. Normal saline was administered to the first group and it was set as control (G1). Rats in the second group were injected with D-gal (500 mg/kg, Thomas Bake, India) for 40 days (G2). Those in the third group received *R. ribes* orally (60 mg/kg, G3). Finally, both D-gal and *R. ribes* were administrated to the rats in the fourth group (G4). The animals were sacrificed after 40 days of D-gal injection and the *R. ribes* administration. In the next step, blood serum was separated for the biochemical study, and the pancreas was taken for immunohistochemical analysis.

Brain and pancreas of rats were subsequently fixed in 10% formalin and then dehydrated in a gradient of ethanol (70, 80, 95 and 100%) prior to being embedded in paraffin wax. Tissue sections were cut at 6 mm thicknesses (Man *et al.*, 2016) and then stained with the immunohistochemical kit.

## RESULTS AND DISCUSSION

Blood serum of the D-gal group (G2) indicated a significant increase in serum glucose, melanoaldihide and beta-amyloid (P≤0.01), however, there was a significant drop in glutathione when compared to other groups. G4 revealed a significant increase in serum glucose, melanoaldihide and beta-amyloid, and a significant decrease in glutathione when compared to the other groups. There was no significant difference between G1 and G3 (Fig. 1). The serum beta-amyloid value rose significantly in the G2 treated group, compared to the other groups (P≤0.01). On the other hand, G4 exhibited a substantial rise in glucose level (P≤0.01) when compared to G1 and G3, but there was no noticeable difference (P>0.01) between G1 and G3 (Fig. 2).

The immunohistochemical study indicated a strong positive stain in the brain and pancreas of the D-gal group as marked by a deep brown stain in Figs. 1, 2, 3, 4, 5, 6, 7 and 8. On the other hand, G4 showed a very weak positive stain for the immunohistochemical assay, compared to G2 (Figs. 6 and 8). A negative result was revealed in G1 and G3.

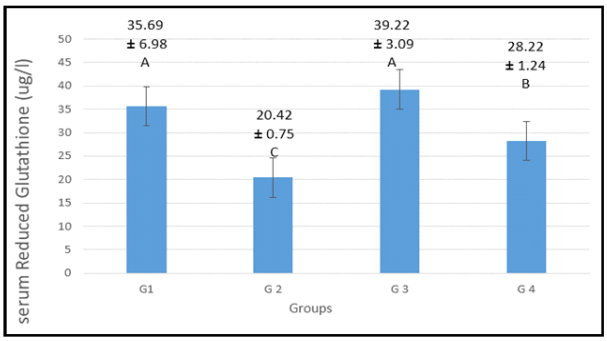


Fig. 1. Effect of D-gal, *Rheum ribes* and their max on serum reduced glutathione (µg/l) in male rats.

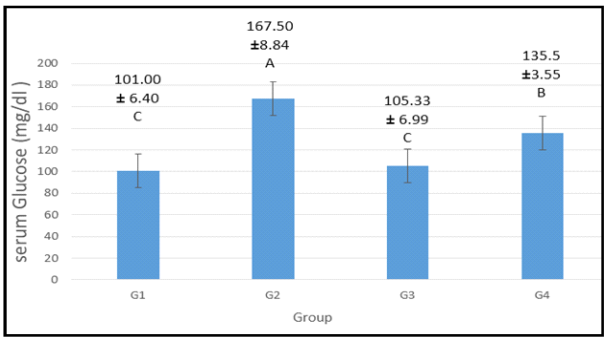


Fig. 2. Effect of D-gal, *Rheum ribes* and their max on serum glucose (mg/dl) in male rats.

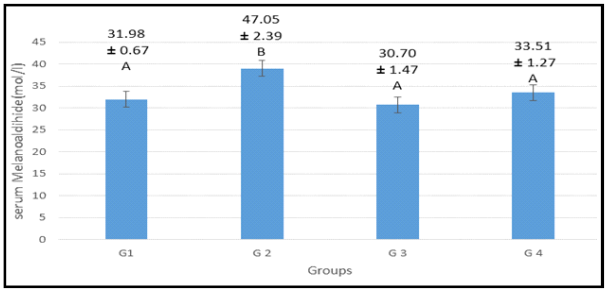


Fig. 3. Effect of D-gal, *Rheum ribes* and their max on serum melanoaldihide (mol/l) in male rats.

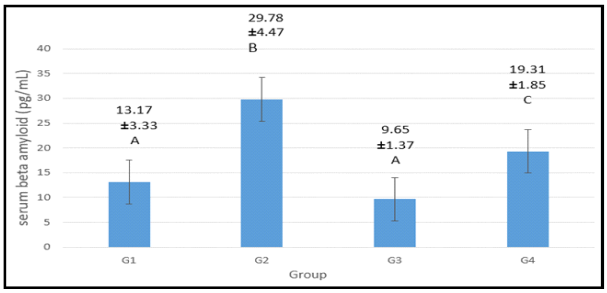


Fig. 4. Effect of D-gal, *Rheum ribes* and their max on serum beta-amyloid (pg/ml) in male rats.

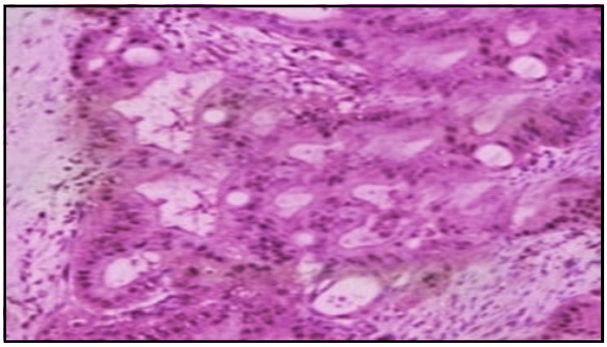

Fig. 5. An immunohistochemical photograph of the pancreas in a rat injected with D-galactose showed the affected area with stains in brown colour.

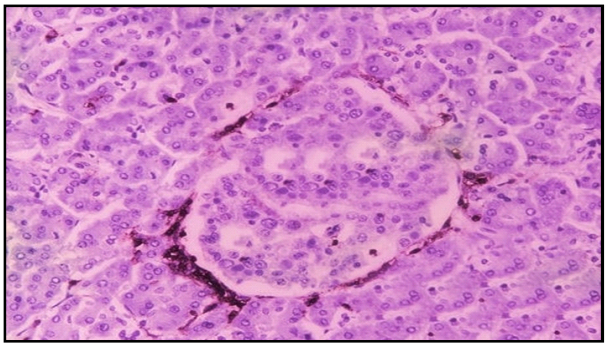

Fig. 6. An immunohistochemical image of the pancreas in a rat with D-galactose injection and *Rheum ribes* administration showed the affected area as a weak positive stain of brown hue colour.

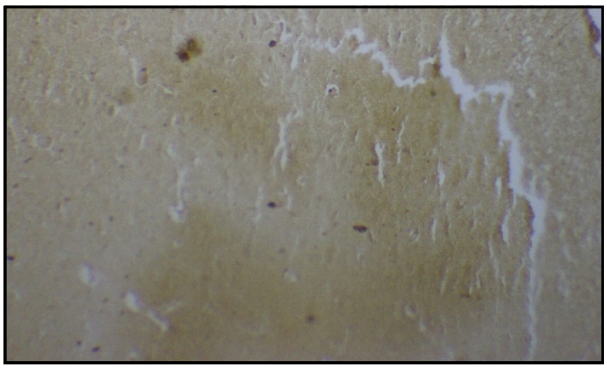

Fig. 7. An immunohistochemical image of the pancreas in a rat injected with D-galactose illustrated the affected area with stains in brown colour.

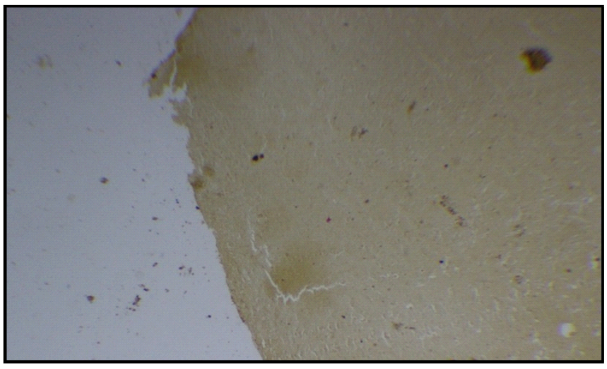

Fig. 8. Immunohistochemical image of the brain in rat that injected D-galactose and administrated *Rheum ribes* indicated the influence region, which appears as a mild positive smear of brown colour.

In the present research, female rats treated with D-gal had a significant rise in glucose levels. Similarly, Thamkaew *et al.* (2021) believed that D-galactose was now known to be an inducer of aging chemicals that could be replicated in aging-related models. It had the potential to harm a variety of organs, including the pancreas (Xinag *et al.*, 2020). When D-gal was given to rats, it caused pancreatic aging due to oxidative stress, which damaged the mitochondrial inner membrane lipids, protein and mitochondrial RNA. This affected mitochondrial function, increased blood glucose and reduced pancreatic insulin production, which was also confirmed by Omidi *et al.* (2020). The reason was that its oxidative characteristics increased melanoaldihide and decreased glutathione levels (Dong *et al.*, 2017). On the other hand, the combination group showed a significant decrease in the levels of glucose and melanoaldihide and an increase in the glutathione level. The hypoglycemic effect of plant extracts might be related to the

presence of insulin-like compounds in plants, such as flavonoids or other materials, which induced Beta-cell regeneration and reactivation to produce more insulin. Cell proliferation can be started as a consequence of beta cell regeneration leading to a significant decrease in the number of Alpha and Delta cells (Qu *et al.*, 2016). The high oxidative stress that occurs due to D-gal injection increased the damage to the nervous system, and consequently, increased the beta-amyloid production from the nerve cells (Imran *et al.,* 2020). Additionally, the obtained results of some studies indicated that *R. ribes* had antioxidant characteristics and this might be the reason for a decrease in melanoaldihide and increased glutathione in the combination group (Dizaye *et al.*, 2019).

## CONCLUSION

In conclusion, *Rheum ribes* had been used as a traditional Turkish treatment for diabetes and hemorrhoids. In Bitlis, the herb was also utilized as a digestive and appetizer. The decoction extract of *Rheum ribes* roots was found to have considerable blood sugar-reducing activity in D-galactose-induced diabetic mice in the current investigation. In healthy mice, however, this extract had no hypoglycemic effect.

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