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Study of the Protective Effect of English pea (*Pissum Sativum L*) Seed Hydroethanolic Extract on Red Blood Cell Membrane Stability in Blood Samples Exposed to Sulfasalazine

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Abstract: *In this laboratory experimental study we assessed antioxidant effect of pea (Pissum sativum L)extract on RBC membrane stability. For this purpose, human blood samples were used in our study and exposed to different doses of extract in blood cells that were previously exposed to sulfasalazine. ANOVA used to analyze the data. Our results showed that administration pissum sativum L extract (2,4,6,mg/kg) led to increase in RBC membrane stability and neutralized the effect of sulfasalazine.*

Keywords: *Pissum Sativum L, RBC, Membrane stability*

1. Introduction

Pea (*Pissum sativum L*) is one of the world's oldest domesticated crops. It's area of origin and initial domestication lies in the Mediterranean, primary in the Middle East. Prior to cultivation, pea together with vetch, vetchling and cheakpeas was part of the everyday diet of hunter-gatherers at the end of the last ice age in the Middle East and Europe. Grain legumes were fundamental crops at the start of the "agricultural revolution" which facilitated the establishment of permanent settlement. Pea was the original model organism used in Mendle's discovery (1866) of the laws of inheritance, making it the foundation of modern plant genetics.[1] RBC membrane consist of the lipid bilayer and skeletal proteins. The proteins have some types,such as spectrin,ankyrin.glicophorin,actin,protein band 3 and protein 4.1. These proteins provide RBC while deformability(an ability to shear stress of the artrial circulation).[4] Spectrin is along ,sytoskeletal and heterodynamic protein composed of modular structure of α and β subunits.Spectrin is crucial for maintaining the stability and structure of the membrane and shape of cell. Spectrin functions as an tetramer protein that cross-links transmembrane proteins,membrane lipids and the actin cytoskeleton,either directly or via adaptor proteins such as ankyrin and protein 4.1.[2][3] Ankyrin is itself connected to a transmembrane protein called "band 3" or anion exchanger protein.This connection is created by protein band 4.2.Ankyrin also has the link with β-spectrin chain. [4] Glycophorin is sialoglycoprotein of cell membrane.It is a membrane-spanning protein and caries suger molecules.It may be involved in the stability and specificity of RBC membrane and classified in sub groups such as A,B and C and most important is A. Glycoforin A consist of 131 aminoacid and 52% carbohydrate.It has the site to link with influenza virus and plasmodium falsiparum.Not with standing people with non-functional glycophorin A have normal RBC appearance.[5][6] Actin or band 5 has the connection with spectrin that is facilitated by protein band 4.1. Actin has the effective influence in protein junction and stability of RBC membrane.[5] Protein band 3 Is atypical polytropical membrane protein and mediates the exchange of the cellular HCO3- with CL- in plasma which has been known as the choloride shift.The N_terminal of protein band 3 has the connection with some proteins such as hemoglobin,protein 4.1,protein 4.2.[5][7] Protein 4.1

stabilizes the spectrin-actin link. It is essential for normal shape and integrity of RBC and provides connection between the skeleton and the plasma.[8]

The red blood cell, as it continuously circulates, must be able to undergo extensive passive deformation and to resist fragmentation.These to essential qualities require a highly deformable yet remarkably stable membrane.The property of membrane deformability determines the extent of membrane deformation that can be induced by a defined level of applied force necessary to allow the cell to pass through capillaries much smaller than the cellular dimensions. Membrane stability, on the other hand is defined as the maximum extent of deformation that a membrane can undergo which it cannot recover completely it's initial shape, the point at which it fails. Normal membrane stability allows erythrocytes to circulate without fragmenting, while decreased stabiliting can lead to cell fragmentation under normal circulatory stress.[9]

An antioxidant is a molecular stable enough to donate electron to a rampaging free radical and neutralize it. Thus reducing it's capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. These low-molecular weight antioxidants can safety interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. Although there are several enzymes system within the body that scavenge the radicals,the principle micronutrient(vitamins)antioxidants are vitamin E(α-tocopherol),vitamin c(ascorbic acid)and β-caroten.[10]

Intracellular GSH appears to be a sensitive indicator of cells overal health , and it's ability resist toxic challenge by (-SH) thiol-disulphide exchange .Various oxygen radical stress have been shown to result in GSSG formation and short term depletion of GSH. Reduced glutathione is also capable of directly scavenging radicals and peroxides by being oxidized to either GSSG or to a mixed disulphid, thereby preventing cell membrane lipid peroxidation, hemolysis and subsequent deleterious effects of cellular function. The reducing power of GSH is the key to multiple actions of GSH at the molecular and cellular levels and to it's effectiveness as a systemic antioxidant.[11]

Vitamin E is believed to be involved in a variety of physiological and biochemical functions. The molecular mechanism of these functions is believed to be mediated by either the antioxidant action of the vitamin or by it's action as a membrane stabilizer. Alpha-tocopherol is an efficient scavenger of lipid peroxyl radicals and hence , it is able to break peroxyl chain propagation reactions. The unpaired electron of the tocopheroxyl radical thus formed tends to be delocalized rendering the radical more stable.The radical form may be converted back to alpha-tocopherol in redox cycle reactions involving coenzyme Q. The regeneration of alpha-tocopherol from it's tocopheroxyloxyl radical greatly enhances the turnover efficiency of alpha-tocopherol in its role as s lipid antioxidant.[12][13]

Flavonoid is polyphenollic substance with antioxidant properties. It is found in different vegetables an fruits. There are some research to investigate the effect of several pure flavonoids, such as Kaempferol, Quercetin, morin and rutin on RBC hemolysis and evaluate their –SH capacity as a indicator of membrane protection , and shown that flavonoid has the effective influence on RBC membrane stability.[14]

Studies have revealed that several herbal derives drugs have been demonstrated to contain principles that possess ability to facilitate the stability of biological membranes when exposed to indusced lyses.Some of these include extracts of Lennea coromandelica, Gmelina asiatica, Gynandropis gynendra and so on[15].

2. Materials and Methods

In this labratory experimental study,male and female human blood samples were divided to control groups and groups exposed to 2,4 and 6 mg⁄kg⁄body weight of hydroalcoholic pissum sativum L extract.In each groups 5 blood samples of 5 people were examined membrane stabilizing activity of each blood samples was calculated and the data were analyzed using ANOVA.

3. Results

Our results showed that sulfasalazine had destabilizing effect on RBC membrane stability (Figure I). However, pissum sativum L extract (2,4,6,mg/kg)led to increase RBC membrane stability and neutralize the effect of sulfasalazine. (Figure II)

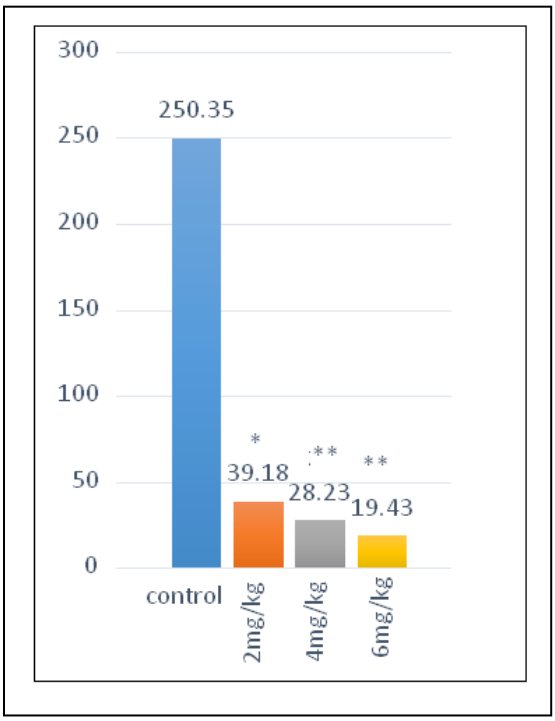

Figure I. Comparison of RBC membrane stability in control groups (Normal salin recipient) and different dosage of sulfasalazine receiving groups (P<0.05 *, P<0.01**)

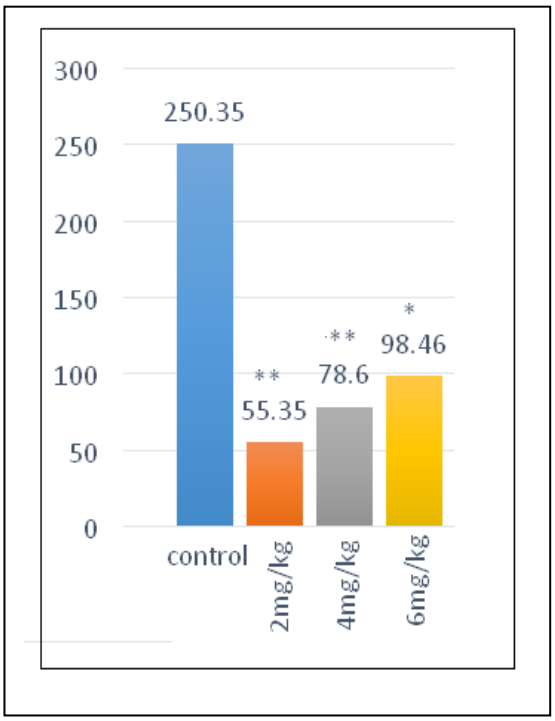

Figure II. Comparison of RBC membrane stability in control groups (Normal salin recipient) and different dosage of extract receiving groups (P<0.05 *, P<0.01**)

4. Discussion

In line with our study, there are studies showing that sulfasalazine decreases antioxidant enzymes e.x golotathion reductase. So sulfasalazine has the effect on raising free-radicals and RBC membrane-lipid defect. [16] One of these, is production of lipid peroxidant that decreases membrane mobility and transmembrane remission defect [10] Therefore, sulfasalazine has the negative effect on RBC membrane stability. In one research the effect of pissum sativum L on colitis which created by DSS (Dextrane Sodium Solphate) has been studied, and showed that pissum sativum L and its albumin part extract is BBI (Bowman-Birk Inhibitor) that care the harms of DSS.[17]

5. Conclusion

Our findings showed that *Pissum sativum L* extract has improving effects on RBC membrane sability.

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7. References

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