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ROLE OF FLAVONOIDS IN THE TREATMENT OF HEMOLYTIC ANAEMIA-A REVIEW

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ABSTRACT

Hemolytic anaemia is caused by premature destruction of erythrocytes either in blood vessels (intravascular) or elsewhere in the body (extravasular). Hemolysis is associated with oxidative stress within the RBCs. A number of antioxidants have been reported to be effective in the treatment of hemolytic anaemia. Flavonoids are phenolic compounds with well known anti-oxidant activity. This review summarizes the research carried out on the various plants containing flavonoids and showing beneficial effects against Phenylhydrazine induced Anaemia.

KEYWORDS: Hemolytic Anaemia; Oxidative Stress; Antioxidants; Flavonoids; Phenylhydrazine.

INTRODUCTION

Anaemia is a medical condition in which the oxygen carrying capacity of blood decreases. It mainly results due to the reduction in number of Red Blood Cells or reduction in the amount of Hemoglobin present.[1] Anaemia may occur due to a number of reasons:

1. Drug toxicity
2. Blood loss
3. Parasites
4. Genetic or acquired defects
5. Lack of nutrition or poor supply of iron
6. Pathological diseases
7. Psychological changes

Some common types of anaemia are:

1. Blood loss Anaemia

If a person losses blood in conditions like hemorrhage or severe injury, the individual is unable to absorb sufficient amount of iron form the intestines to form Hemoglobin Therefore the RBCs formed are smaller than normal leading to microcytic, hypochromic anemia.

2. Aplastic Anaemia

Bone marrow aplasia is the lack of functioning of bone marrow. It may be occur due to excessive exposure to radiations, chemicals and even drugs.

3. Megoblastic Anaemia

Due to the poor absorption of Vitamin B_{12}, folic acid and intrinsic factors from the stomach mucosa there is slow production of erythroblasts in the bone marrow leading to oversized RBCs with odd shapes and fragile membranes called the megloblasts. These cells rupture easily leading to anaemia.

4. Hemolytic Anaemia

A number of abnormalities of RBCs make the cells fragile resulting in reduced life span leading to serious anaemia.[2]

Hemolytic Anaemia may be classified as

a) Congenital or inherited hemolytic anaemia

The oxygen carrying capacity and life span of RBC is reduced due to genetic abnormality in the synthesis of hemoglobin and increased membrane friability. Sickle Cell Anemia and Thalassemia are the most common forms of inherited hemolytic anemia.

b) Acquired hemolytic anaemia

It may occur due to a number of reasons:

- **Chemical agents** causing early or excessive hemolysis e.g: lead or arsenic compounds, certain drugs when taken in large amount, toxins produced by microbes
- **Autoimmunity:** In this case the immune system of an individual makes antibodies against its own red cell antigens causing hemolysis
- **Blood Transfusion Reactions**: If a person receives blood carrying antigen different from their own, their immune system makes antibodies against the antigen and destroy them.
- **Parasitic diseases**: e.g: malaria
- **Ionizing radiations**
- **Physical damage to cells** e.g: artificial heart valves, kidney dialysis machines.[3]

RELATIONSHIP BETWEEN OXIDATIVE STRESS AND HEMOLYTIC ANAEMIA

Oxidative stress is the main reason involved in the occurrence of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, diabetes, hypertension, hypoxia, ischemia reperfusion injury and heart failure.[4,5] Vascular pathology involves a number of reactive species e.g. reactive oxygen and nitrogen species among which the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) appear to be the most important. Increased $O_2^{\cdot-}$ formation is probably a major cause of accelerated inactivation of NO and induction of endothelial dysfunction.,[6,7,8] Because of the Loss of NO bioactivity normal functioning of the cardiovascular, hematological and immunological systems is affected.[9]

A number of researches have been carried out the study the mechanism of hemolytic actions of several agents on RBCs and it has been reported that hemolytic injury is associated with oxidative stress within erythrocytes. This concept is supported by the fact that hemolytic damage is accompanied by the generation of reactive oxygen species (ROS), glutathione depletion, hemoglobin (Hb) oxidation and Heinz body formation in RBCs. Hemolytic agents have been reported to cause membrane lipid peroxidation and denaturation of cytoskeletalprotein.[10]

Experimental hemolytic anemia has been reported to be treated by some anti-oxidant drugs such as flavonoids. It has also been demonstrated that the antioxidants such as flavonoids can act:

- Either by neutralizing ROS by directly reacting with superoxide anion, NO and peroxynitrite thereby preserving vascular function and protecting vascular injuries from ROS and perhaps from other oxidant species, including phenyl hydrazine (PHZ) radicals.
- Or the**y** could stimulate erythropoiesis process.[11]

ROLE OF FLAVONOIDS IN ANAEMIA

Flavonoids are naturally occurring plant phenolics and biologically active secondary plant metabolites with beneficial human effects. They are mainly found in plant derived foods especially in the skin of fruit and epidermis of leaves. They have reported anti-inflammatory, anti-oxidant, anti-allergic, heptoprotective, ant-thrombotic, anti-viral and anti-carcinogenic activities.[12]

Flavonoids are known to possess a well-established protective effect against membrane lipoperoxidative damages.[1] The antioxidant activity of phenols and flavonoids is mainly attributed to their redox properties because of which they act as reducing agents, electron/hydrogen donators and singlet oxygen quenchers. In addition, they have a metal chelating potential.[13] The unique chemical structures of phenolic compounds is characterized by an aromatic ring possessing one or more hydroxyl substituent and is predictive of their antioxidant potential in terms of radical scavenging, hydrogen- or electron-donating and metal-chelating capacities.[14]

EXPERIMENTAL INDUCTION OF HEMOLYTIC ANAEMIA BY PHENYLHYDRAZINE

Phenylhydrazine is a strong oxidant, which is extensively used as an antipyretic in therapeutic settings, industry or laboratory. The various toxic effects of PHZ that have been reported are hemolytic anemia, hypoxia, inflammation, alterations in the liver, kidney, central nervous system, autoimmune disturbances and cancer. It is known to shorten the lifespan of RBCs resulting in severe hemolytic anemia, increased iron absorption and tissue iron over-loading.[11] Its oxidation causes generation of reactive oxygen species (ROS) and generation of a complex number of derived radicals, such as phenylhydrazyl radical, phenyldiazene and benzenediazonium ions. It is known to induce Heinz body formation and oxidative degradation causing erythrocyte deformability.[15] Formation of phenyl radicals and the replacement of heme with phenyl-substituted protoporphyrins, causes the destabilization of Hb to induce Heinz bodies and hemolytic anemia with PHZ.[16]

Pretreatment of the rats with the flavonoid-rich extracts prior to Phenylhydrazine treatment effectively blocked the effect of PHZ-induced malondialdehyde damage to RBC membrane glycerol back-bone and peroxidation of phospholipids.[17]

PLANTS REPORTED TO CONTAIN FLAVONOIDS AND SHOW ANTI-ANAEMIC POTENTIAL

A number of plants have reported to show anti-anaemic activity in phenylhydrazine induced anaemia due to the presence of flavonoids:

1. Magniferra indica

Phytochemical screening of the extracts revealed the presence of saponins, flavonoids and alkaloids. Thus, it appears that the presence of these antioxidants in the plant extracts reverses the damaging effect of phenyl hydrazine. A study on *M. indica* showed that mangiferin, a normal metabolite in the plant's leaves and stem, had strong antioxidant property.[18]

2. Telfairia occidentalis

Phytochemical screening of leaves extract of **T. occidentalis** showed the presence of flavonoids, saponins, cardiac glycosides and alkaloids. The Leaves extract were reported to possess significant antianaemic potentials due to the presence of various antioxidants.[19]

3. Schrebera swietenioides

In this study, significant decrease in parameters like Hb, RBC count and hematocrit was observed following PHZ injection to the experimental animals. Treatment with different doses of *S. swietenioides* root bark extract resulted in increased values of these parameters. The Hb concentration was found to be higher than the positive

control animals. This indicated that the root bark contains some bioactive agents that are powerful antioxidants, which removed or repaired the damage done to the cells by free radicals or highly reactive oxygen species. A significant observation was that the content of polyphenols, flavonoids and tannins was much higher in Root Extract compared to Leaf Extract and Stem Extract. The anti-anemic property of *S. swietenioides* could be attributed to the presence of the above-mentioned bioactive as they are known to exert anti-oxidant activity as reported in the literature. From the study, it could be established that the anti-anaemic potential of Root Extract could be explored for further research in developing a novel herbal delivery system.[20]

4. Entandrophragma angolense

Phytochemical analysis revealed the presence of large chemical groups including alkaloids, tannins, flavonoids, polyphenols, quinones, sterols, terpenes, cardiac glycosides, saponins and leucoanthocyanins. They all have antioxidant power, promote regeneration of tissue, reduce the permeability of blood capillaries and increase their resistance to hemolysis. The presence of these chemicals by their properties justified the resistance of red blood cells of treated rats with the extract. Flavonoids are known to possess anti-anaemic potential and veinotonic properties, which protects the blood capillaries. The injection of phenylhydrazine to rats caused a hemolytic anemia characterized by reducing hematological parameters. The oral administration of aqueous and ethanol extracts *of Entandrophragma angolense* in the dose of 200 mg / kg / day significantly increased haemoglobin level in the first week of treatment. The anti-anaemic potential of the plant was suggested to come from phytochemicals and also the possible vitamin and mineral constituents.[21]

5. Jussiaea repense

Erythrocytes of control, *Jussiaea repense* supplement and *Jussiaea repense* treated groups showed normal biconcave shape with no such morphological alterations. This might be due to protective nature of JR extract containing various anti-oxidant metabolites i.e. rutin, kaempferol, quercetin etc. Quercetin have been reported to play an important role against oxidative damage with preservation of erythrocyte membrane integrity.[22]

6. Mukia maderaspatana and Kredrostis foetidissima

Both the plants of curcurbitaceae family have anti-anaemic activity in different potentials. The difference in anti-anaemic potentials of the plant extracts is due to the difference in the concentration of phytoconstituents especially flavonoids.[23]

7. Spinicia Oleracea

In the study of *S. oleracea* leaves, the phytochemical investigation indicated the presence of flavonoids, saponins, cardiac glycosides, terpenes, steroids and resins. The study provided scientific evidence regarding the efficacy of *Spinacia oleracea* extract in the management of anaemia in phenylhydrazine-treated rats. The haematinic potential of the plant was suggested to be due to the phytochemicals which increase the haematological indices (PCV, Hb and RBC) and also by vitamin and mineral constituents of the plant needed in the restoration of normal haematological conditions.[24]

8. Moringa oleifera

The Plant have been reported to contains a number of flavonoids, saponins, triterpenes, steroids, alkaloids and many other chemical constituents. In extract-treated groups, there was also a significant increase in the parameters when compared with the control and anaemic groups. This could be due to the phytochemical constituents in the extract and also presence of minerals and vitamins. These constituents are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow.[25]

9. Jatropa tanjorensis

The Phytochemical screening of leaves showed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinines and saponins. The presence of these antioxidants in the plant sample was suggested to be responsible for the reversal of damaging effect caused by phenylhydrazine on RBCs.[26]

10. Silybium marianum

Silymarin refers to the extract from the seeds of the plant *Silybum marianum*, also called "milk thistle". Silymarin may be an effective "antioxidant," which means milk thistle may be helpful in fighting oxidation. Flavonoid silybin present in silymarin has been reported to protect RBCs from haemolysis by preventing the generation of free radicals and maintaining the membrane integrity of the RBCs thus preventing the haemolysis by counteracting PHZ induced damage.[11]

11. Carissa Edulis

The ethanolic root bark extract revealed the presence of phytochemicals such as flavonoids, tannins and terpenes which are known to show anti-oxidant activity. The presence of these phytoconstituents in the *Carissa edulis* extract were suggested to be responsible for the faster and dose-dependent reversal of anaemia compared to normal saline group.[27]

12. Wrightia Tinctoria

Extracts of *W. tinctoria* bark were subjected to phytochemical screening out of which methanol extract, methanolic aqueous fraction, and water extract were found to contain more number of anti-anaemic phytochemical such as carbohydrates, tannins, phenolic compound and flavonoids. The aqueous fraction of *W. tinctoria* bark methanolic extract showed rich presence of flavonoid and polyphenolic compounds. Extract showed good hematopoietic activity possibly due to the high flavonoid content (7.66%) as well as the sandwich

effect of other phytoconstituents present in aqueous methanolic fraction of *W. tinctoria.*[1]

CONCLUSION

Though a number of drugs are available for the treatment of anaemia they are not affordable to many poor people especially those in the developing countries where anaemia is very common. In addition, the rural populations in various parts of the world depend mainly on plants and herbal products for treatment on any kind of disease as do not have adequate access to high quality drugs. As anaemia is very common and its incidence is most likely to increase in future, there is need to prevent it or seek for new affordable therapies which are easily accessible and which are cost effective.

Therefore by the means of this review article it can be shown that Flavonoids have been extensively reported by many researchers to play an important role in the treatment of Anaemia. It can further be suggested that Flavonoid containing plants can serve as a better alternative for the treatment of anaemia as flavonoids are abundantly found in nature.

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