

INHIBITORY EFFECT OF ARECANUT EXTRACT ON H₂O₂ INDUCED RBC HAEMOLYSIS

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Introduction

Reactive Oxygen Species (ROS) mediated oxidative damage to macromolecules was implicated in the pathogenicity of major diseases such as cancer, rheumatoid arthritis, post-ischemic reperfusion injury, degeneration process of ageing, myocardial infarction, cardiovascular disease etc. Their accumulation during ageing leads to loss of function in various tissues.

Erythrocytes (RBC) acts as an oxygen carrier and it has shorter life span and is susceptible to oxidation stress during circulation due to high content of polyunsaturated lipids, their rich oxygen supply and the presence of transition metals such as iron and copper. RBCs are threatened by free radicals generated within the cell. Erythrocytes, as a single cell, (RBCs) have been used for a model to investigate oxidative damage in biomembranes. H₂O₂ produced in the aqueous phase attacks the erythrocyte membrane to induce oxidations of lipids and proteins and eventually cause haemolysis. It was found that ageing individuals showed changes in the properties of the erythrocyte membrane and the rate of haemolysis.

Hydrogen peroxide is a small molecule that easily passes through all membranes of the cell.

H₂O₂ is generated through non-enzymatic or enzymatic dismutation of superoxide. On the other hand, it can be further oxidized to form hydroxyl radical (OH). Among the various forms of activated O₂, OH radicals are most reactive and therefore most destructive to the cells and tissue organs in biological system and formation of hydroxyl radicals from H₂O₂ and O₂ is greatly facilitated by Fe. H₂O₂ can damage many cell components such as DNA, lipid and protein either directly or indirectly.

Arecanut is obtained from fruit of the *Areca catechu* palm. The major biochemical components of arecanut are arecoline (0.15%), polyphenols (20%), fats (15%), polysaccharides (20%), fibre (8%) and protein (9%). Arecanut extract showed potent antioxidant activity and inhibition of free radicals and reactive oxygen species. Arecanut possesses antidiabetic, anti-depressant and anti-microbial properties. This study aimed to evaluate the antioxidant activity of arecanut protection on erythrocyte oxidative haemolysis in young and aged rats.

Blood was obtained from rats and collected into EDTA contained tubes. RBC was separated

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from plasma by centrifugation at 2000 rpm for 20 min. Crude RBC was then washed three times with 5 volumes of phosphate buffered solution (PBS). 10 % RBC was prepared by 1 ml RBC suspended in 9 vol of PBS.

1ml RBC was mixed with 1 ml of PBS solution containing various amounts of Ethanol extract of Arecanut (1 ml). The H₂O₂ (0.44%) solution (1 ml) was then added to the mixture. RBC suspension (1 ml) mixed with 1 ml of H₂O₂ solution alone was used as control sample. The incubation mixture was shaken gently in a water bath at 37 °C for 1 h. After incubation, the mixture was centrifuged at 2000 rpm for 10 min. Absorbance (A) of the supernatant at 540 nm was recorded with a UV-visible recording spectrophotometer.

Percentage inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = \frac{(\text{OD H}_2\text{O}_2 - \text{OD Arecanut extract})}{\text{OD H}_2\text{O}_2} \times 100$$

Where OD *Arecanut extract* is the absorbance of the sample containing arecanut extract and OD H₂O₂ is the absorbance of the control sample. Experiments are done in triplicate and values are average of two experiments.

RESULTS

Erythrocytes have polyunsaturated fatty acids, molecular oxygen and ferrous ions since erythrocytes would be highly vulnerable to oxidative stress. However, these cells have an efficient antioxidant system, which makes them resistant

to peroxidation when radicals are produced within the cell.

When the content of active oxygen species exceeds the cellular antioxidant capacity, irreversible damage to RBC takes place, resulting in haemolysis and removal from the circulation.

The present work investigated the peroxidation of the erythrocyte membrane, in which free radicals were generated by an exogenous source. The peroxidation of erythrocyte membranes can be studied with a variety of agents, including hydrogen peroxide, dialuric acid, xanthine oxidase and organic hydroperoxides.

The results are presented in table 1. The ethanol extract from arecanut was evaluated for its effect against hydrogen peroxide (H₂O₂) induced oxidation in young and old rat erythrocytes. RBC preincubated with increasing amounts of arecanut extract and then incubated with H₂O₂ was analyzed for haemolysis. Ethanol extract of arecanut exhibited a strong protection for RBC haemolysis 78% inhibition at 50 µg concentration and 65% inhibition and 100 µg of arecanut extract. In high concentration 500 µg and 1000 µg has also showed inhibition of haemolysis 57% and 58% respectively. But in case of young rats arecanut extract in low concentration was found to have inhibitory effect where as high concentration exhibited haemolysis

It was observed that arecanut extract high concentration, which inhibited haemolysis in aged rat, showed haemolysis in the young rats. This finding is also in accordance with the earlier evidences, which state that membranes from aged

animals respond very different than do membranes from young animals. It has been reported that membrane order of hepatic microsomal membrane decrease with age. Free iron contents of old RBC from donors were much higher than those of young RBC. A phase transition was detected for membranes of aged rats but not for younger rats.

The important activity compounds in arecanut ethanol extract mainly contain polyphenol. A series of dimeric, trimeric and tetrameric procyanidins have recently been isolated from the seeds of *Areca Catechu*. The polyphenols, mostly flavonols, include about 10 per cent of (+) catechin, 2.5 per cent epicatechin, 12 per cent of (+) leucocyanidin, the remaining portion being complex flavonoids in varying degrees of polymerization. The crude phenolics extract of whole areca fruit contained 92 mg/g of condensed tannins, 69 mg/g hydrolysable tannins, 84 mg/g of non-tannin flavonols and 56 mg/g of simple phenolics.

Procyanidins are a class of polyphenolic polymers composed of flavan-3-ol units (catechin and epicatechin) Proanthocyanidins are a special class of flavonoids exhibiting potent antioxidant properties. Recent studies have shown that proanthocyanidin in grape seeds possess anti-inflammatory, anti-arthritic and anti-allergic activities and prevent heart disease and skin ageing.

Previous studies reported the polyphenols in green tea as epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG) and

gallic acid(GA) were able to suppress the erythrocyte hemolysis from AAPH-oxidation.

Similarly, Palm fruit crude extract (CE) and ethanolic extract (EE) protected RBC from AAPH-induced haemolysis in a dose dependent manner. Palm fruit extracts reduce the rate and extent of oxidation of haemoglobin exposed to oxidative stress.

It has also been observed that *in vitro* studies on flavanols and the procyanidin oligomers from cocoa exhibited dose-dependent protection against 2,2'-azo-bis (2-amidinopropane) dihydrochloride (AAPH)-induced erythrocyte haemolysis. Plasma antioxidant capacity (as measured by the total antioxidant potential [TRAP] assay) was elevated following the cocoa extract feeding. Erythrocytes obtained from the cocoa extract-fed animals showed an enhanced resistance to haemolysis.

In another study, four epicatechin isomers purified from jasmine tea exhibited a strong protection for RBC membrane to haemolysis induced by 2,2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH). The inhibitory effect was dose-dependent.

The mechanism of inhibition of haemolysis showed by arecanut ethanolic extract might be polyphenolic compounds directly scavenge H_2O_2 or peroxy radicals. Various studies have shown that phenolic antioxidant capability derives from the ability of the phenolic group to donate a hydrogen atom to a chain-propagating free radical, neutralizing it, and forming a more stable free

radical or complex that then reacts in nonchain-propagating way. Another possibility is that polyphenol stabilize the erythrocyte membrane by suppressing the loss of glycolytic intermediates, such as intracellular ATP (adenosine triphosphate) and 2,3-DPG (2,3-diphosphoglycerate). Flavanols and polyphenols interact with membrane phospholipids through hydrogen bonding to the polar head groups of phospholipids which might act to maintain membrane integrity. Further studies should be carried out to identify the compounds

and precise role in prevention of oxidative haemolysis of RBC.

CONCLUSION

From this study, we can conclude that ethanolic arecanut extract has an activity on protection of erythrocytes that is possible to be affected by radicals such as H_2O_2 or peroxy radical. Further research on ageing and membrane to determine the extent to which alterations in membrane composition and action due to polyphenol from arecanut may be carried out.

Table 1: Arecanut extract inhibition of haemolysis on aged and young rats RBCs

Concentration of Arecanut extract (μ g)	% haemolysis	% Inhibition
Old Rats		
10	21.64	52.06
25	47.94	70.08
50	29.92	78.36
100	34.09	65.91
250	44.60	55.40
500	43.00	57.00
1000	41.54	58.40
Young Rats		
50	30.69	69.31
500	60.12	39.88
700	69.98	30.05
