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COMPARISON OF ANTIOXIDANT EFFECT OF GLUTATHIONE AND ASTAXANTHINE IN THEIR THERAPEUTIC RANGE USING IN- VITRO METHODS

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ABSTRACT

Now a days ,free radicals and antioxidants seek a great deal of attention. Free radicals are produced naturally in our body by various endogenous reactions. However at higher concentrations, they can cause cell membrane , DNA and protein damage leading to various immunopathological states like inflammatory diseases, cancer hypertension, diabetes mellitus, atherosclerosis, ageing etc .An antioxidant is a stable molecule which donates its electron to a rampaging free radical and thus neutralizes its effect. Imbalance between free radicals and antioxidants leads to a condition called oxidative stress. Antioxidants can be endogenous or exogenous. Various dietary antioxidant supplements are available commercially. The objective of this study is to compare the antioxidant effect of glutathione and astaxanthine in their therapeutic range using in- vitro methods

KEYWORDS :

INTRODUCTION

Free radicals are molecular species with an odd or unpaired number of electrons and are formed when oxygen interacts with certain molecules.Free radicals are formed constantly in human system either as accidental products during metabolism or deliberately during the process of phagocytosis.. They can also be generated from environment, pollutants, ozone, smoking , and chronic alcohol intake.Once formed, these highly reactive radicals can start a chain of reaction and can oxidise biomolecules leading to cell membrane , DNA and protein damage .They also play important role in the immunopathology of a lot of inflammatory diseases,cancer, hypertension,diabetes mellitus, ageing atherosclerosis, etc. An antioxidant is a stable molecule which donates an electron to a rampaging free radical and thus neutralizes its effect- free radical scavengers. When an imbalance occurs between free radicals and antioxidants, oxidative stress occurs leading damage to our body system.Though some antioxidants are formed endogenously, our body also relies on exogenous sources. These exogenous antioxidants may be from diets or from dietary supplements. Now a days the use of antioxidant dietary supplements is on the rise. Many preparations are available commercially. Glutathione and astaxanthine are commonly prescribed antioxidants by doctors. The objective of this study is to compare the antioxidant effect of glutathione and astaxanthine in their therapeutic range using in- vitro methods.

Here two invitro methods are used to compare their antioxidant activities

1)**FPAP assay**

MATERIALS AND METHOD

1.Test drug : 1. Crude drug of Glutathione(500mg)
2. Crude drug of Astaxanthine(8mg)

2.Standard: Ascorbic acid

3.Reagent: FRAP reagent

Total antioxidant activity is measured by ferric reducing antioxidant power assay of Benzie and Strain (1999). The FRAP assay, is presented as a novel method for assessing "antioxidant power." The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy. The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids within the technological reach of every laboratory and researcher interested in oxidative stress and its effects.

PRINCIPLE:

Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form. There is no apparent interaction between antioxidants.

Reagents:

Reagent A: Acetate Buffer (300 mM, pH 3.6):

16 ml of glacial acetic acid was added to 3.1g of sodium acetate trihydrate. The solution was then made up to 1 litre using distilled water. The pH of the solution was checked using pH meter.

Reagent B: TPTZ solution:

0.031g of TPTZ was added to 10 ml of 40 mM HCl and dissolved at 50◦C.

Reagent C: Ferric chloride solution:

0.054g of ferric chloride was dissolved in 10 ml of distilled water

The working FRAP reagent was prepared by mixing A, B & C in the ratio of. 10:1:1 at the time of use.

Procedure:

*100 µl of sample was added to the tube marked test and 3 ml of FRAP reagent was added to it.

*3 ml of FRAP reagent was taken as a blank.

*Absorbance is measured at 0 minutes after vortexing at 593 nm.

*Samples are then placed at 37°C in water bath and absorption is again measured after 4 minutes.

*Ascorbic acid was used as the standard.

S.No.	Contents	FRAP ASSAY	
		Blank	Test
1.	Sample	-	100µl
2.	Working FRAP Solution	3ml	3ml

Calculation:

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute / change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000 µM)

Note: FRAP value of ascorbic acid is 2.

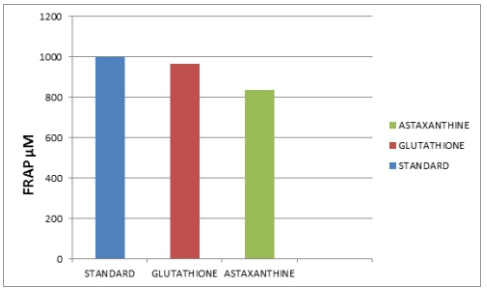
Result:

Table 1 : Comparison of the Antioxidant activity of the given samples using FRAPAssay

S.NO	Name of the sample	FRAP(µM)
1.	Glutathione	965
2.	Astaxanthine	835

Change in absorbance of Glutathione from 0 to 4 minute = 0.167
Change in absorbance of Astaxanthin from 0 to 4 minute = 0.193
Change in absorbance of Standard from 0 to 4 minute = 0.2

Figure1:Bar diagram comparing the antioxidant activities using FRAP assay



2) SCAVENGING OF NITRIC OXIDE RADICALS

MATERIALS AND METHODS

1.Test sample: 1.crude drug of glutathione (500mg)
2.crude drug of astaxanthine(8mg)
2.Reference antioxidant: Ascorbic acid
3.Solvent: sodium nitroprusside in phosphate buffer
4.Reagent: Griess reagent
5.Spectrophotometer

Principle:

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitrite oxide which interacts with oxygen to produce Nitrite ions, which can be measured at 550nm by spectrophotometer in the presence of Griess reagent (Kumar S et al., 2008).

Procedure:

Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) was incubated with 100 mg/ml of sample and tubes were incubated at 29ºC for 3 hours. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagent. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthyl ethylene diamine hydrochloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to sample.

Calculations

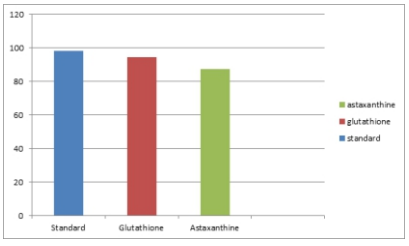
$$\% \text{ inhibition} = \frac{\text{O.D. of control} - \text{O.D. of Test}}{\text{O.D of control}} \times 100$$

RESULTS:

Table2: Comparison of percentage of inhibition of glutathione and astaxanthine with standard

S.NO	NAME OF THE SAMPLE	INHIBITION%
1.	Glutathione	94.30
2.	Astaxanthine	87.41
3.	Standard	98.5

Figure 2:Bar diagram comparing the percentage inhibition



CONCLUSION

From the above invitro methods we can conclude that glutathione has a better antioxidant effect than astaxanthine with in their therapeutic range. However further invitro studies and invivo studies are needed to support our results.

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