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Original Article

Anti-Oxidant Action of Curcumin – An In-Vitro Assessment

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ABSTRACT

Objectives: To prove the efficacy of Curcumin (C) as an effective anti-oxidant using Fenton's reagent (FR) as an inducer of oxidative stress by generation of Reactive oxygen species (ROS) in an in-vitro experiment.

Methods: The blood samples collected were divided into 3 groups:

Group 1: Control - plasma and serum, Group 2: FR+ (plasma and serum) incubated for 1 hour at 37^0 C with Fenton's Reagent only, Group 3: C+/FR+ (plasma and serum) incubated with Curcumin for 1 hour followed by incubation with Fenton's reagent for 1 hour at 37^0 C

The samples were then analyzed for Malondialdehyde (MDA), Ischemia modified albumin (IMA), Uric acid and albumin levels and the difference in their values in the three groups were statistically analyzed.

Results: Fenton's reagent brought about oxidative stress, indicated by a rise in MDA and IMA and decrease in uric acid and albumin levels. The anti- oxidant protective effect of Curcumin was reflected by a decrease in IMA and MDA and increase in uric acid levels in Group 3 samples. The effect on albumin was inconclusive.

Conclusion: The in-vitro experiment helped to establish the role of Curcumin as a potent anti-oxidant with significant efficacy.

KEYWORDS: Albumin, Anti-oxidants, Curcumin, Fenton's reagent, Ischemia Modified Albumin, Malondialdehyde, Uric acid.

INTRODUCTION

Curcumin is a flavonoid Diferuloylmethane obtained from the plant Curcuma longa, a common Indian spice used in kitchen. Since ages the anti-inflammatory and anti-septic property of curcumin is known to people and generally used for the same in Indian households. In recent times, the anti-oxidant and anti-cancer properties of Curcumin is being evaluated and studied extensively to establish it as a potent anti-oxidant and anti-cancer agent with comparative efficacy and ample range of safety. Its rife availability, efficacy and safety makes it a potential therapeutic candidate for cancer .Hence, in-vitro and in-vivo studies to establish its anti-oxidant and anti-tumour activity is an active area of research now.

Oxidative stress induced DNA mutation and lipid peroxidation is linked to ageing and development of many dreaded diseases like cancers, central nervous system disorders and cardiovascular diseases.[1] Metabolic processes of the body generates the free radicals exposing the body to continuous oxidative stress .Albumin and uric acid are considered to be the body's natural defence system against oxidative stress.[2,3] whereas plasma Malondialdehyde (MDA) is a marker of lipid peroxidation due to oxidative damage.[4] Recently, serum Ischemia modified albumin (IMA) is also measured as marker of oxidative damage to proteins though the aetiology of IMA is ischemia induced conformational change of albumin.[5]

Fenton's reagent consists of Hydrogen peroxide with ferrous sulphate as catalyst which generates oxidative free radicals and induces oxidative stress related damage to biomolecules like lipids proteins and nucleic acids.[6] Fenton's reagent is normally used for purification of water by oxidative damage to biological wastes.[7]

Reactive hydroxyl radical (OH) can be artificially produced in vitro by the Fenton-reaction using Fenton's reagent:

1. $Fe2+ + H2O2 \rightarrow Fe3+ + HO\bullet + OH-$
2. $Fe3+ + H2O2 \rightarrow Fe2+ + HOO\bullet + H+$

(Fenton reaction)

Curcumin, a flavonoid derived from the Indian spice Curcuma longa has proven anti-septic and anti-inflammatory properties but recently it is being extensively studied for its anti-cancer and anti-oxidant properties by various in vitro and in vivoexperiments.[8-11]

The advantage of Curcumin as a therapeutic anti-oxidant and anti-cancer agent is its wide and easy availability, safety and cost-effectiveness. Hence, more studies on Curcumin proving its role as an effective anti-oxidant will be greatly beneficial to the therapeutic arena and regular use of Curcumin as therapy to prevent oxidative damage to the body can be advocated with better exactitude. The beneficial effects of curcumin as an antioxidant and its underlying mechanism of action was investigated by Miao et al. in diabetic rat model and their conclusion was that curcumin could notably improve the antioxidant ability of the diabetic model resulting in an improvement in the metabolic disorder.[12] Rai et al.[13] showed that curcumin expresses its anti-cancer activities by increasing levels of anti-oxidants like vitamins C and E, and preventing lipid peroxidation as indicated by serum MDA level and DNA damage. In another study by Asori et al.[14] it was documented that curcumin is a potent anti-oxidant comparable to Ascorbic acid in prevention of damage induced by free radicals. DeSilvestro[15] in their study showed that lipid soluble extract Curcumin was capable of lowering the serum triglyceride level. They also documented that Curcumin increased the level of anti-oxidant enzyme catalase and decreased the level of liver injury marker, alanine transferase.

Venkata et al.[16] established a strong affinity of curcumin to various inflammatory mediators like ERK, PKC, P38 MAP Kinase, NFkB and Lipoxygenase and came to the conclusion that Curcumin can be used as an effective molecule for treatment of various inflammatory disorders owing to its multi-target potency and wider safety profile.

With this knowledge a study was done in the Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha with an aim to prove the efficacy of Curcumin as a potent anti-oxidant using Fenton's reagent as an inducer of oxidative stress by generation of ROS (reactive oxygen species) in an vitro experiment.

MATERIALS AND METHODS

The ethical approval was obtained from institutional ethical committee. After written consent, 30 healthy medical students in the age group of 17-25 years were enrolled for the study. Students having immediate history of any infectious diseases, medicine intake, vitamins and anti-oxidant supplementation, family history of any chronic / genetic diseases, any addiction to tobacco/alcohol were excluded from the study. 10 ml of blood sample was obtained from the participants and serum and plasma were separated immediately and the samples were stored in 4-8^0 C until analysis.

Fenton's reagent was freshly prepared with 30% H_2O_2 (Hydrogen peroxide) and 1mmol $FeSO_4$ (Ferrous Sulphate) in the laboratory just before use. Strength of Curcumin used was 200µmol after standardisation.

Each sample was divided into 3 parts:

Group 1: Only serum/plasma

Group 2-: Serum /plasma incubated with Fenton's reagent in the ratio 100µl per ml of sample at 37^0 C for 1hour.

Group 3: Serum/plasma incubated with Curcumin in the ratio 100µl per ml of sample for 1 hour followed by Fenton's reagent in the ratio 100µl per ml of sample for 1hour at 37^0 C

Each group were then analyzed for serum Ischemia modified albumin by colorimetric assay using Dithiothreitol (DTT)[17] plasma Malondialdehyde by Thiobarbituric acid (TBA) method[18], serum albumin by Bromocresol green (BCG method) and serum uric acid by enzymatic method.

Data obtained from the three groups were statistically analyzed by SPSS 20 to see the significance of the obtained results.

RESULTS

Group 1 shows the baseline MDA, IMA, albumin and uric acid values of the study group. Group 2 shows the values of the same parameters estimated after the serum/plasma sample were incubated with Fenton's reagent (30% H_2O_2 +1mmol $FeSO_4$) for I hour at 37^o C. Group 3 shows the observed MDA, IMA, albumin and uric acid levels in the serum/plasma samples after they were first incubated with Curcumin (200µmol) for 1hour at 37^o C followed by Fenton's reagent for 1hour at 37^o C. Our study documented a rise in IMA and MDA levels after incubation with Fenton's reagent which was statistically significant ($p<0.001$) for MDA but not so for IMA.

There was a decrease in the levels of albumin and uric acid in group 2 compared to group 1which was also statistically significant <0.001)

Table 1: Show the observations obtained in the study groups and parameters.

STUDY GROUPS	SERUM MDA nmol/ml Mean ±SD	PLASMA IMA ABSU Mean ±SD	SERUM ALBUMIN gm% Mean ±SD	SERUM URIC ACID mg% Mean ±SD
1-serum/plasma	0.90±0.39	0.36±0.14	4.4±0.36	5.5±2.1
2-serum/plasma+ FR	1.24±0.56*	0.41±0.19	3.7±0.47*	3.1±1.1*
3 serum/plasma + Curcumin + FR	0.95±0.40**	0.30±0.17**	3.7±0.35	4.3±1.4**

***p<0.001 between group 1&2, ** p<0.01 between group 2&3**

After incubation with Curcumin followed by Fenton's reagent in group 3, there was a significant decrease in serum IMA and MDA levels ($p<0.01$).Similarly, our study registered a significant rise in serum uric acid level in group 3 compared to group 2 ($p<0.01$). However, we didn't observe any significant change in serum albumin level in group 3 compared to group 2 levels.

When Group 1 levels (baseline) of IMA and MDA were compared with Group 3 levels, no statistically significant difference was noted reflecting the protective effect of Curcumin on the biomolecules from oxidative stress induced by Fenton's reagent.

DISCUSSION

Oxidative stress and free radical induced damage is a continuous process occurring in human body. Physiological process like ageing and most of the chronic diseases is attributed to oxidative stress and free radical induced damage to cell membranes and nucleic acids consisting of lipids and proteins. Malondialdehyde (MDA) has been used as a marker to assess the oxidative stress induced damage to lipids. Recently, Ischemia modified albumin (IMA), is considered to reflect the free radical damage to proteins like albumin though the major causative factor behind generation of IMA is ischemia which ultimately induces oxidative stress. Albumin and uric acid are considered to be the natural defence system of the body against oxidative stress due to their anti-oxidant properties.

Fenton's reagent contains Hydrogen peroxide which, in presence of a catalyst like ferrous sulphate generates free radicals. Our study utilized this property of Fenton's reagent to generate free radicals in-vitro and induce oxidative damage to biomolecules like lipids and proteins reflected by the levels of MDA, IMA.

Curcumin, the active ingredient of Indian spice *Curcuma longa*, a rhizome, possess anti – inflammatory and anti-septic properties tried and tested since ages. In recent times, more research is being done on its anti- oxidant and anti- tumour activity.

In our study, we estimated the baseline levels of MDA, IMA, albumin and uric acid in the blood samples. We reanalyzed the samples for the same parameters after incubating the samples with Fenton's reagent (100µl per ml of sample) which generated free radicals and observed a rise in MDA and IMA levels (Group 2 vs. Group 1) substantiating the fact that free radicals damage the lipids and proteins which is indicated by the rise in MDA and IMA levels. Our observations corroborated with other studies which have documented a rise in MDA and IMA level by free radical induced oxidative damage.[11, 19-22]

Uric acid and serum albumin are effective natural anti-oxidants that the body possess and its decreased level is registered in many studies associated with oxidative stress.[2,3,23,24] There was a definitive decrease in the levels of albumin and uric acid in Group 2 compared to Group 1 in our study, which proved that Fenton's reagent used in the required amount and concentration effectively caused oxidative stress induced damage which shaped an easy and effective in-vitro model to be used to test the efficacy of new potential anti-oxidants.

The protective anti-oxidant property of Curcumin was established in our study by the decreased level of MDA and IMA after incubation with Curcumin, which was statistically significant. Curcumin efficiently showed a scavenging effect against free radicals by preventing the generation of MDA and IMA. Similarly, the natural anti-oxidant of the body, uric acid showed a rise after incubation with Curcumin in Group 3 which indicated that uric acid was spared by Curcumin to counter the damage induced by Fenton's reagent which is the novelty of our study. However, our observation on serum albumin was inconclusive.

CONCLUSION

The in-vitro experiment helped in proving the efficacy of Curcumin as a potent anti-oxidant and its role in scavenging the free radical induced generation of IMA and MDA. Fenton's reagent was successfully used to generate free radicals indicated by a rise in MDA and IMA level and a fall in natural anti-oxidants of the body uric acid and albumin. The sparing effect of Curcumin on uric acid was also documented while effect on albumin was inconclusive.

Further studies with bigger sample size and in vivo interventional studies will help in providing indisputable evidence in this regard and help in wider acceptance of Curcumin as a natural, economical and safe anti- oxidant in clinical scenario.

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