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Research Article

Inhibition of *In Vitro* Fructose-Induced Protein Glycation *by Litchi chinensis*

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ABSTRACT

Non enzymatic glycation is a chain reaction between reducing sugars and the free amino groups of proteins, involved in severity of diabetes and diabetic complications. *Litchi chinensis* used as consumed fruit and as a drug to treat certain diseases. In this study the antioxidative effects of *L.chinensis* and also its effect against protein oxidation and advanced glycation end products. The antioxidant potential of aqueous fruit pericarp extract of *L.chinensis* (APLC) was evaluated *in vitro* using a model of fructose-mediated protein glycation. The antioxidant activity of *APLC* conducted for superoxide, hydroxyl, hydrogen peroxide, nitric oxide radical scavenging activities and also demonstrated antioxidant activity with Fe+2 chelating activity, ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) were applied. Fructose (100mM) increased fluorescence intensity of glycated bovine serum albumin (BSA) in terms of total AGEs during 21 days of exposure. Moreover, fructose caused more protein carbonyl (PCO) formation in native BSA. The *APLC* prevents oxidative protein damages including effect on PCO formation which are believed to form under the glycoxidation process. The *APLC* at different concentrations (25-250µg/ml) has significantly decreased the formation of AGEs in term of the fluorescence intensity of glycated BSA.

Keywords: Advanced glycation end products, Protein glycoxidation *and Litchi chinensis*.

INTRODUCTION

Free radicals are continuously metabolized in the body as the result for metabolic processes and interact with the environmental stimuli. Under normal physiological conditions wide range of antioxidant defense mechanism protect the body against free radicals[1]. In normal conditions, the human body undergoes a number of the physiological and biochemical processes leads to the production of several radicals. The reactive oxygen species are superoxide radical ($O2^{\cdot-}$); hydrogen peroxide (H_2O_2); hydroxyl radical ($HO^{\cdot}$); peroxyl radical (ROO-) and hypochlorous acid (HOCl) (Sanchez-Moreno C., 2002). The reactive nitrogen species (RNS): nitric oxide ($^{\cdot}NO$) and peroxynitrite ($ONOO^{-}$) which are formed by the reaction of $^{\cdot}NO$ and superoxide during inflammatory processes[2]. The overproduction and the imbalance between the radical species (ROS, RNS) and the capacity of the normal detoxification systems which are represented by enzymatic and nonezymatic antioxidants lead to the generation of oxidative stress. It has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of a number of human diseases[3].

The accumulation of the reaction products of protein glycation (nonenzymatic reaction of proteins with glucose and other reducing sugars) in living organisms leads to structural and functional modifications of tissue proteins[4]. Free radicals can induce protein modifications including losses of protein functions such as the activity of enzymes, receptors, and membrane transporters, resulting in biological dysfunctions[5] by formation of Amadori intermediates[6,7] and finally leads to the formation of advanced glycation end products.

Medicinal plants are frequently considered to be less toxic and free from side effects than the synthetic ones. Protective effects of natural antioxidants have been studied *in vitro* and *in vivo* glycation models in recent years, thus providing beneficial effects of antioxidants mainly from plant sources[8].The increased demand for the use of plant based medicines in the treat diabetes may be due to the side effects caused by commercially available orthodox drugs such as insulin and oral hypoglycemic agents[9].

Both synthetic compounds and natural products have been evaluated as AGE inhibitors. Although some synthetic compounds demonstrated strong inhibitory activities against the formation of AGEs or in breaking protein cross links caused by maillard reaction *in vivo*, they may also lead to severe side effects. As an example, aminoguanidine, the first AGE inhibitor engaging in clinical study, was terminated in phase III clinical trial in diabetic patients due to safety concerns[10,11]. Earlier so many plant based extracts were reported for *in vitro* glycoxidation and inhibition of advanced glycation end products are *Astragalus Radix*[12], *Teucrium polium*[13], *Vitis vinifera*[14] *Mesona Chinensis*[15], *Salvia reuterana*[16] and *Feronia limonia*[17]. Hence in the present study was carriedout with *Litchi chinensis* for *in vitro* glycoxidation

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and inhibition of advanced glycation end products. The fruit pericarp extract of *Litchi chinenesis* (Sapindaceae) consists of anthocyanins like cyanidin-3-glucoside, cyanidin-3-diglucoside, cyanidin- 3-rutinoside[18], malvidin- 3-glucoside and quercetin-3-rutinoside[18], pelargonidin 3, 7- diglucoside, rutin and epicatechin[19]. The phenolics of litchi pericarp have been confirmed to have antioxidant, anticancer[20], immunomodulatory[19] activities. Litchi fruit pericarp has been considered a new source of pharmaceuticals and food industry. Hence in the present study the aqueous pericarp extract of *Litchi chinensis* was evaluated for its i*n vitro* antioxidant and inhibition of protein glycoxidation activities.

MATERIALS AND METHODS

Materials

Nitroblue tetrazolium (NBT), bovine serum albumin (BSA) were obtained from Merck, Mumbai. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 4 dinitrophenylhydrazine (DNPH), trichloroacetic acid (TCA) were obtained from Sigma (St. Louis, MO, USA). 2.4.6-Tri- (2′-pyridyl)-1, 3, 5-triazine (TPTZ), 2, 2′-Azinobis- (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) and Trolox were obtained from Sigma Aldrich Chemical Co., Ltd. (England). All other reagents were of analytical reagent (AR) grade.

Plant material preparation

The ripened litchees *(Litchi chinensis*) were obtained from local market. The peels were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of pericarp powder was found to be 13.5%. The powder was suspended in 2% gum acacia and used in the experimental studies.

Super oxide radical scavenging activity

The assay was based on the capacity of the aqueous extract to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light- NBT system[21]. The reaction mixture contained 58 mM phosphate buffer, pH 7.6, 20µM riboflavin, 6mM EDTA, and 50µM NBT, final volume made up to 3 ml, added in that sequence. Initiated with the reaction [22] the reaction mixture with the different concentrations was exposed to 40 volts under fluorescence lamp for 15min to intiated the reaction. Immediately after illumination, the absorbance was measured at 560 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes, with reaction mixture, above were kept in the dark and served as blanks. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \text{ x } 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the aqueous extract/standard. All experiments were performed in triplicate.

Hydroxyl radiacal scavenging activity

Scavenging activity of hydroxyl radical was measured by the method of Halliwell et al., 1985[23]. Hydroxyl radicals were generated by a Fenton reaction ($Fe3^+$-ascorbate-EDTA-H_2O_2 system), and the scavenging capacity of the extract and standard towards the hydroxyl radicals was measured by using deoxyribose method. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations (10-1000 µg/ml) of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \text{ x } 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch et al., 1989[24]. The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 43mM H_2O_2 was prepared in 0.1M phosphate buffer (pH 7.4). The APLC of different concentrations were prepared in 3.4mL phosphate buffer were added to 0.6mL of H_2O_2 solution (43mM) and absorbance of the reaction mixture was recorded at 230 nm. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \text{ x } 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

DPPH radical scavenging activity

The potential of extract and AA was determined on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1ml of a methanolic solution containing each concentration of extract were added to 3ml of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated[25]. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample. All tests were run in triplicate and averaged.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [26]. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and APLC and the AA in different concentrations were incubated at 25°C for 150 min. After incubation 1.5ml of the Griess reagent (1% sulphanilamide and 0.1% naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

Reducing power

The reducing power of the extract was determined according to the method of Oyaizu et al., 1986[27]. Various concentrations of the extracts (mg/ml) in distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 ml, K_3 [Fe $(CN)_6$]). The mixture was incubated at 50ºC for 20 min. Aliquots of trichloracetic acid (2.5 ml, 10% aqueous solution) were added to the mixture which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared FeCl3 solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

Phosphomolybdinum method

The antioxidant activity of *APLC* extract was evaluated by the phosphomolybdenum method of Prieto et al., 1999 [28]. An aliquot of 0.1 ml of sample solution (equivalent to 100 lg) was combined with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In the case of the blank, 0.1 ml of methanol was used in place of sample. The tubes were capped and incubated in water bath at 95^0C for 90 min. After the samples were cooled to RT, the absorbance of the aqueous solution of each was measured at 695 nm.

The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

Fe^{+2}Chelating activity

The chelating activity of the extracts for ferrous ions (Fe^{2+}) was measured according to the method of Dinis *et al.,* 1994[29]. To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) was added. After 30 sec, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the $Fe^{2+/}$Ferrozine complex was measured at 562 nm. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =

$$\frac{A_O - A_1}{A_O} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

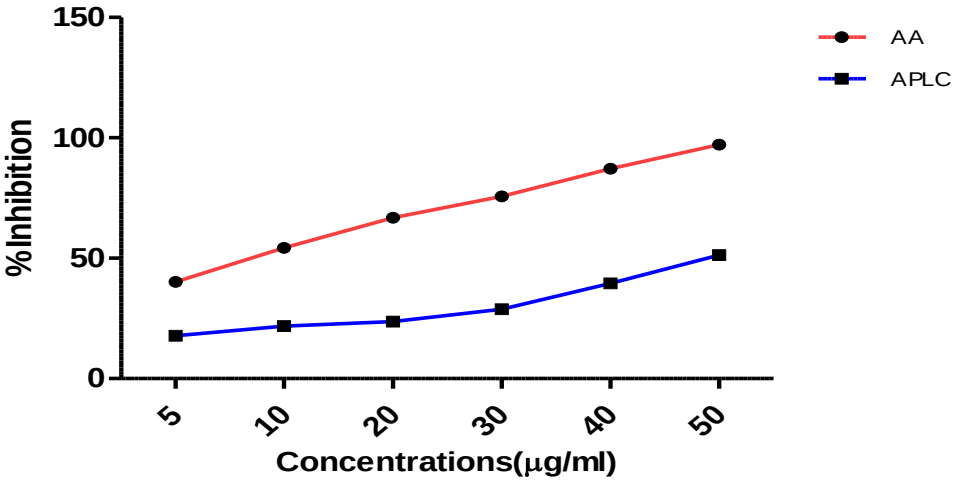
Ferric reducing ability power

The FRAP method measures the absorption change that appears when the TPTZ (2,4,6 -tri pyridyl-*s*-triazine)-Fe^{3+} complex is reduced to the TPTZ-Fe^{2+} form in the presence of antioxidants[30]. An intense blue colour develops with absorption maximum at 595 nm. The FRAP reagent contained 2.5 ml of 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6, was freshly prepared. The extracts were dissolved in ethanol at a concentration of 1 mg/ml. An aliquot of 0.2 ml of solution was mixed with 1.8 ml of FRAP reagent and the absorption of the reaction mixture was measured at 595 nm. Ethanolic solutions of known Fe (II) concentration, in the range of 50-1000 µM (FeSO4), were used for obtaining the calibration curve (Graph 2). The FRAP value represents the ratio between the slope of the linear plot for reducing Fe^{3+}-TPTZ reagent by plant extract compared to the slope of the plot for $FeSO_4$.

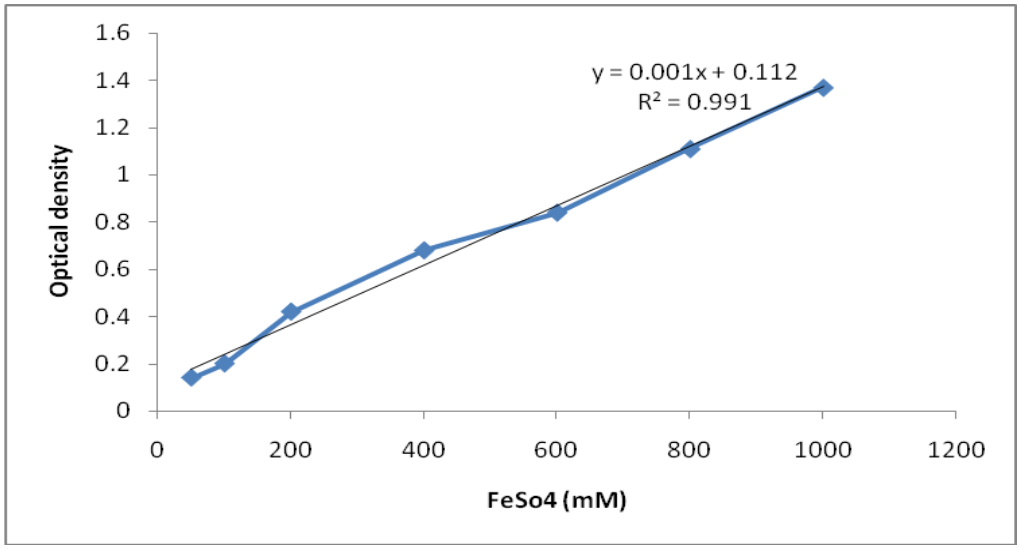
ABTS Assay

The ABTS assay was based on the method of Re *et al.,* 1999[31] with slight modifications. ABTS radical cation ($ABTS^+$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS+ solution was adjusted to an absorbance 0.70±0.02 by diluting with ethanol at 734 nm. The 25µl of sample or standard Trolox was added to 2 ml of diluted $ABTS^+$ solution, and the absorbance was

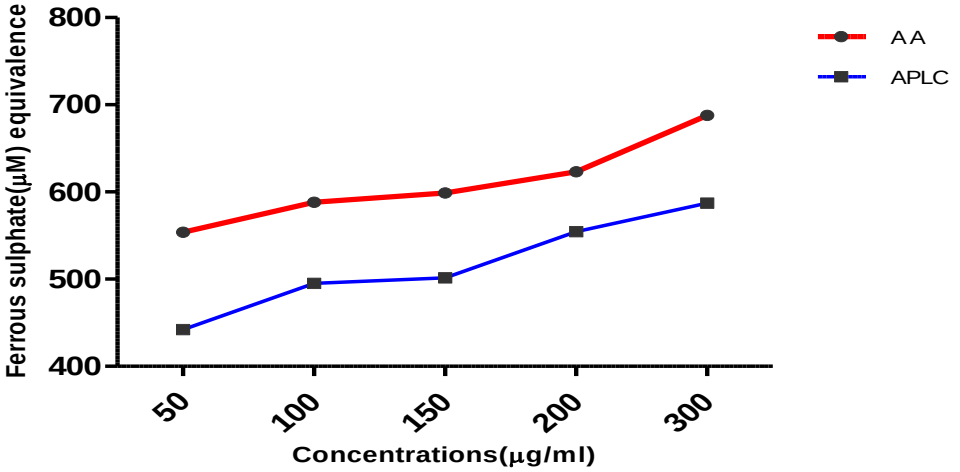
measured after 6 min. The decrease in absorption with the addition of different concentrations of extract was used for calculating TEAC values. A standard curve was



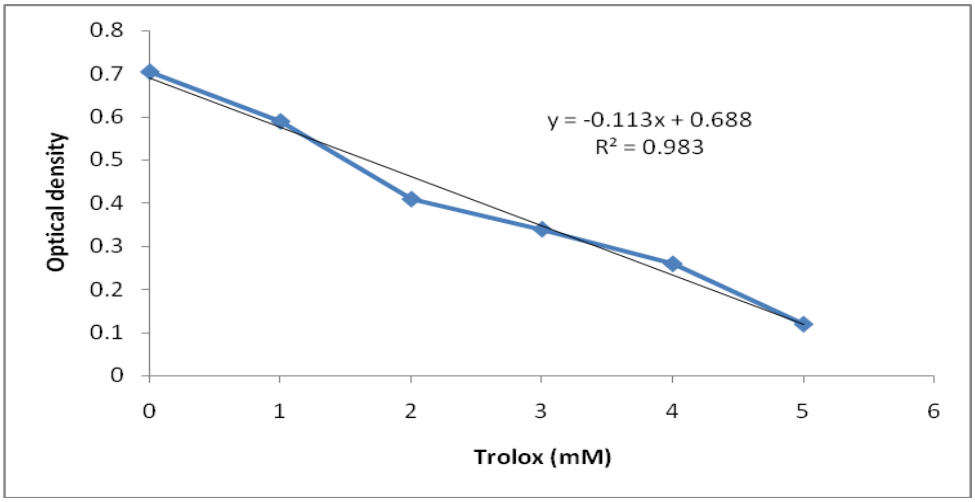
Graph 1: Effect of APLC on Fe^{+2} Chelating Activity.



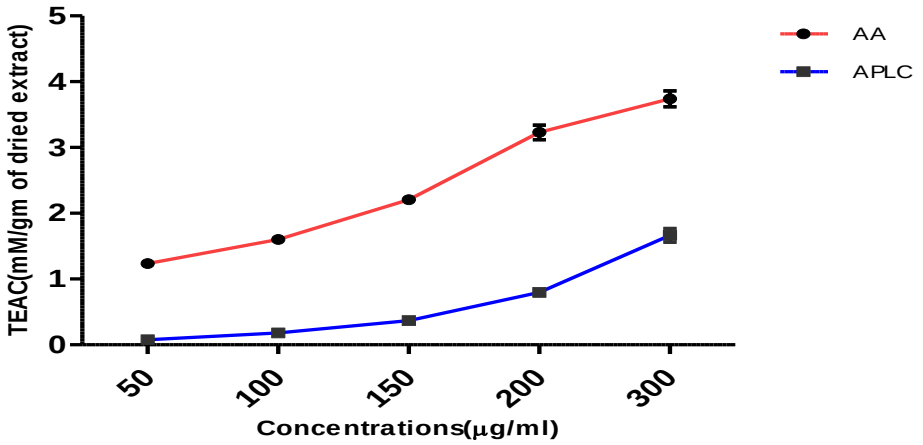
Graph 2: Standard graph of ferrous sulphate.



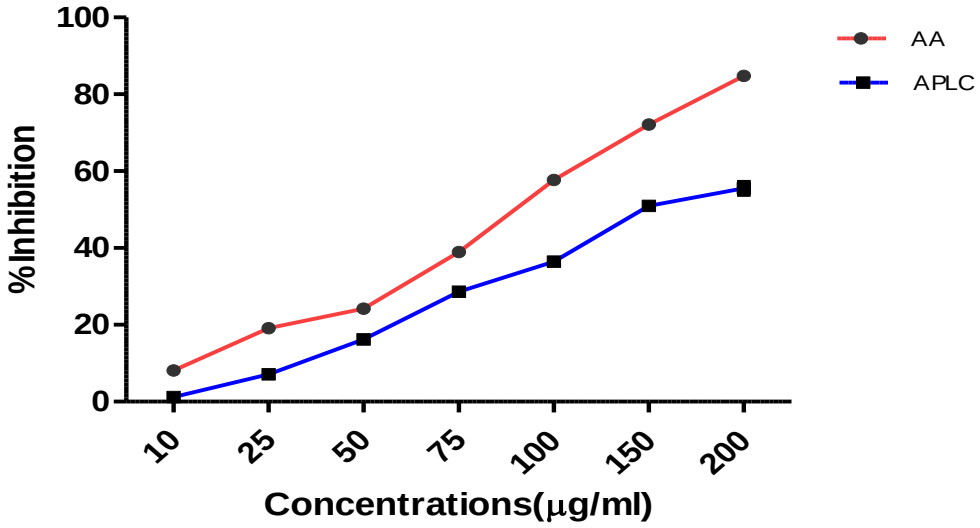
Graph 3: Effect of APLC on Ferric reducing antioxidant power (FRAP)



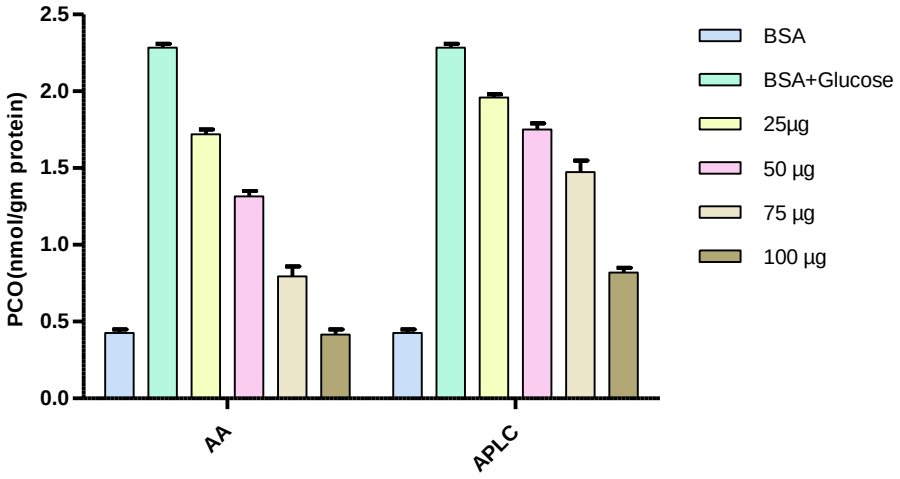
Graph 4: Standard graph of Trolox



Graph 5: Effect of APLC on ABTS radical scavenging assay:



Graph 6: Effect of APLC on *in vitro* glycoxidation:



Graph 7: Effect of APLC on Protein carbonyl content.

prepared by measuring the reduction in absorbance of $ABTS^{+}$ solution at different concentrations of Trolox (Graph 4). Appropriate blank measurements were carried out and the values recorded. The reduction in the absorbance of different concentrations of extract was measured from the trolox standard graph a TEAC values. Results were expressed as Trolox equivalent antioxidant capacity (TEAC).

In Vitro protein glycoxidation method

This assay was adopted from the literature by Wu & Yen, 2005 [8] and used as an *in vitro* model. In brief, 5 g BSA and 14.4 g D-glucose were dissolved in phosphate buffer (1.5 M, pH 7.4) to obtain the control solution with 50 mg/mL BSA and 0.8 M D-fractose. 2 mL of the control solution was incubated at 37^0C for 21 days in the presence or absence of 1 mL of APLC and AA in phosphate buffer (1.5 M, pH 7.4) (the final concentration of extract in the 3 mL test solution was 500 ppm). The test solution also contained 0.2 g/L NaN_3 to assure an aseptic condition. After 21 days of incubation, fluorescent intensity (excitation, 370 nm; emission, 440 nm) was measured for the test solutions. Percent inhibition of AGE formation by APLC and AA was calculated using the following equation,

% inhibition = [1 (fluorescence of the solution with inhibitors/Fluorescence of the solution without inhibitors)] ×100%.

Estimation of protein carbonyl content

The effects of *APLC* extract on oxidative modification of BSA during glycoxidation process were carried out according to method Ardestani and Yazdanparast, 2007[32]. For determination of protein carbonyl content in the samples, 1 ml of 10 mM 2, 4-dinitrophenylhydrazine (DNPH) in 2 M HCl was added to the samples (1 mg). Samples were incubated for 30 min at RT. Then, 1 ml of cold TCA (10%, w/v) was added to the mixture and centrifuged at 3000g for 10 min. The protein pellet was washed three times with 2 ml of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 ml of guanidine hydrochloride (6 M, pH 2.3). The absorbance of the sample was read at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH. The data were expressed as nmol/mg protein**.**

RESULTS

Antioxidant activity of APLC using different in vitro assay systems

Several assays have been explained for the measurement of free radical scavenging activity of pericarp extract including superoxide radical, hydroxyl radical, hydrogen peroxide, DPPH radical and nitric oxide radical scavenging activities. The antioxidant capacity was determined by reducing power assay and phosphomolybdenum assay methods. The AA and APLC showed IC_{50} values of 4.77±0.31 and 8.58± 0.21µg/ml respectively in scavenging superoxide radical. The extract was found to be moderate scavenger of superoxide radical generated in riboflavin-NBT-light system *in vitro.* Hydroxyl radical-scavenging activity of the APLC extract was investigated using the Fenton reaction mechanism; the AA and APLC showed IC_{50} values of 29.7±0.11 and 58.50± 0.03µg/ml respectively in scavenging the hydroxyl radical. The AA and APLC showed IC_{50} values of 5.08±0.03 and 3.60±0.04 µg/ml respectively in scavenging the hydrogen peroxide radical. The APLC and AA showed IC_{50} values of 172.2±4.51 and 53.92±5.22 µg/ml respectively in scavenging DPPH radical. The APLC and AA showed IC_{50} values of 65.77±1.41 and 16.93±0.07 µg/ml respectively in scavenging nitric oxide free radical. The AA and APLC showed IC_{50} values of 5.52±0.01 and 8.61±0.02 µg/ml respectively in reducing power assay. The IC_{50} value of reducing the Mo (V) complexes was 3.19±0.03 and 3.02±0.02 respectively for AA and APLC in phosphomolybdenum assay. The results were presented in table1.

Inhibitory effect of APLC on Fe+2 chelating activity

The intensity of red color complex was disrupted by presence of AA and APLC. The AA and APLC showed IC_{50} values of 8.86±0.13 and 30.58±0.00 µg/ml respectively in Fe+2 chelating activity. The APLC showed a dose dependent effect with an increase in the concentration. The results were presented in graph 1.

Table 1: Effect of Ascorbic acid and APLC on IC_{50} values of different *in vitro* models.

Method	Ascorbic acid	APLC
Superoxide radical	04.27±0.31	08.58±0.21
Hydroxyl radical	29.70±0.11	58.50±0.24
Hydrogen peroxide radical	05.08±0.03	03.60±0.04
DPPH radical	53.92±5.22	172.26±4.51
Nitric oxide radical	16.93±0.07	65.77± 1.41
Reducing power	05.52±0.01	08.61±0.02
Phosphomolybdinum method	03.19±0.03	03.02±0.02

Inhibitory effect of APLC on FRAP assay

The AA and APLC showed inhibitory concentrations of 687.75±0.65 and 694.7±0.35 respectively to Ferrous sulphate (mM) equivalence. The FRAP value of APLC shown to have dose dependent effect with an increase in the concentration. The results were presented in graph 3.

Inhibitory effect of APLC on $ABTS^+$ radical scavenging activity

The TEAC value (graph 5) of APLC was found to have dose dependent effect with an increase in the concentration, the AA and APLC showed TEAC values of 3.74±0.12 and 1.89±0.02 mM/gm dried extract/AA respectively.

Inhibitory effect of APLC on in vitro glycation of BSA

The AA and APLC inhibited the glycation of BSA and subsequent formation of fluorescent glycation products in a concentration dependent manner (graph 6). IC_{50} value for inhibition of AGE were found to be 102.7± 1.73 and 144.2±1.37µg/ml for AA and APLC respectively and the activity was found to be dose dependent with an increase in the concentrations of APLC and AA.

Inhibitory effect of APLC on PCO content

The AA and APLC shown to decrease the formation of PCO in a dose dependent manner (graph 7). The PCO content with 100 µg/ml of AA and APLC was found to be 0.41±0.03 and 0.54±0.02 nmol/mg of protein respectively.

DISCUSSION

The reactive oxygen species are generated in the early and advanced glycation processes. The glycation phenomenon corresponds to the non enzymatic and non-oxidative covalent attachment of glucose molecule to protein. This process classically proceeds through early and advanced stages. In the early stage, reducing sugars such as glucose react with the amino groups of lysine side chains and the terminal amino group of proteins to form unstable Schiff bases and, through rearrangement, Amadori products. Then, this product undergoes slow and complex series of chemical reactions to form advanced glycation end products (AGEs)[33].

Antioxidants works as in various pathways by scavenging the radicals and decomposing peroxides by binding to the metal ions and some of them producing synergistic action. These free radicals act by damaging proteins, DNA, and other small molecules. These antioxidants work by three different mechanisms as antioxygen radicals, reducing substances and antiradicals. They work as metal chelators by chain breaking and reducing the chain initiation. Therefore antioxidant activity can be evaluated by different *in vitro* methods for different mechanisms.

In the present study, we evaluated the antiglycation properties of *Litchi chinensis* pericarp extract against both aqueous, lipid peroxyl free radicals and non enzymatic glycation and oxidation of BSA.

The present study reveals that APLC is a potent scavenger of deleterious free radicals formed during metabolic reactions as well as endogenous free radicals such as O^- and OH^-, at very low concentrations. Superoxide is the first reduction product of molecular oxygen, a highly toxic radical, the most abundantly produced in all aerobic cells by several enzymatic and non-enzymatic pathways, attacks a number of biological molecules and leads to unfavourable alterations of biomolecules including DNA[34]. It also forms an important source of other deleterious radicals such as hydroxyl and hydroperoxides, which initiate free radical chain reactions[35]. The APLC were shown similar activity like AA in scavenging the superoxide and hydroxyl radicals in a dose dependent manner (table 1). Biological systems can produce hydrogen peroxide[36] by several oxidizing enzymes such as superoxide dismutase. It can cross-membranes and may slowly oxidize a number of compounds. The DPPH antioxidant assay is based on the ability antioxidant to decolorize DPPH, a stable free radical, When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance[37]. The APLC showed a significant dose-dependent inhibition of DPPH radical scavenging activity. Nitric oxide free radicals generated from the reactive oxygen free radicals and are also implicated in inflammation, cancer, diabetes and other pathological conditions[38]. The APLC shown to counteract the effect of NO formation by scavenging the nitric oxide free radical *in vitro* (table 1).

In the phosphomolybdinum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes[39]. In the present study APLC was found to have dose dependent radical scavenging activity of Mo (VI) as shown in table 1. Iron is essential for life due to its unusual flexibility to serve as both an electron donor and acceptor can be found as either ferrous (Fe2+) or ferric ion (Fe3+), with the latter form predominating in foods. If iron is free within the cell it can catalyze the conversion of hydrogen peroxide into free radicals[40]. Ferrous chelation may render important antioxidative effects by retarding metal-catalysed oxidation[41]. The reducing power of APLC was found to be beneficial over the standard ascorbic acid as shown in table 1. The Ferric reducing antioxidant power assay takes advantage of an electron transfer reaction such as ferric iron (Fe3+) can facilitate the production of ROS[42]. The ability of APLC to chelate iron can be a valuable antioxidant property. In this assay, APLC inhibits the formation of the ferrous to

ferrozine complex, indicating that APLC has Fe^{2+} chelating activity in a concentration dependent manner (graph 1).

FRAP assay measures the reducing ability of antioxidant that react with ferric tripyridyltriazine ($Fe3^{+}$-TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ)[28] The APLC showed better scavenging activity to reduce ferric ions, reflecting their ability to decrease reactive oxygen species (graph 2, 3). The APLC shown to have effect on scavenging the transition metals catalyzed auto-oxidation of glucose and further on reactive carbonyl compounds to form AGEs. Thus, APLC may retard the process of AGEs by preventing further oxidation of Amadori products and metal-catalyzed glucose oxidation as reported earlier by Rahbar S., 2003[43]. In addition, the APLC was also shown to have scavenged the blue-green colored ABTS+ radical cation relative to the radical scavenging ability of standard Trolox and represented as TEAC values (graph 4, 5).

During the early stage of glycation, Schiff bases are prone to oxidation leads to generating variety of free radicals, reactive carbonyl groups and formation of AGEs. Oxidative modifications of BSA incubated with fructose were demonstrated using a combination of protein carbonyl assay[44]. To evaluate *APLC* can reduce the protein glycation and extent of protein carbonyl formation after 21 days by DNPH reagent. As shown in graph 6, 7 glycation elicited a significant increase of carbonylation of BSA in the presence of fructose compared to the control sample without reducing sugar. However a significant effect on the inhibition of protein oxidation due to glycation was exerted in a dose-dependent manner with increasing the concentrations of APLC.

The APLC exhibited strong antioxidant activity in scavenging the different free radicals. In addition to its antioxidant activity it also showed better activity against the formation of protein carbonyl content and protect the structural changes in BSA during glycation processes. All the activities might be due to high levels of anthocyanins and polyphenols in aqueous pericarp extract of *Litchi chinensis*.

CONCLUSIONS

It is concluded that *Litchi chinensis* possess good antioxidant, antiglycation and inhibition of formation of advanced glycation end products. It may useful in the treatment of several pathological complications produced by advanced glycation end products like diabetes mellitus.

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