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Original Article

Antioxidant Properties of ADJ6 and ITS Effect on Isolated Human Erythrocyte Ghost Membrane

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ABSTRACT

Introduction: Oxidative stress is one of the major causes for many adverse complications especially in disorders such as diabetes, cardiovascular diseases, cancer, etc. Many studies have proven that erythrocytes are affected during these conditions and oxidative stress plays a critical role in damaging the membrane integrity of erythrocytes by inducing lipid peroxidation causing instability to membrane in turn affecting the ion transport mechanisms. **Objective and methods:** The present study focuses to study the ameliorative effect of ADJ6 against oxidative stress on human erythrocytes. The membrane stabilization activity, Inhibition of lipid peroxidation and the effect against Ouabain induced Na^+ K^+ ATPase activity was assessed. Further, *in vitro* antioxidant activity of ADJ6 was also determined. **Results and Discussion:** DPPH radical scavenging activity, metal chelating and H_2O_2 radical scavenging activity were IC_{50}=37.9 ± 0.22 µg/ml, IC_{50}=53.95 ± 0.337 µg/ml and IC_{50}=69.5 ± 0.628 µg/ml respectively. ADJ6 showed an EC_{50}=90.83 ± 1.905 µg/ml against hypotonicity induced lysis of erythrocytes. H_2O_2 induced Lipid peroxidation of ghost membrane was also inhibited by ADJ6 (IC_{50}=84.5 ± 2.613 µg/ml). Ouabain induced sodium potassium ATPase activity increased with the treatment of ADJ6 in a dose dependent manner. This proves that ADJ6 is effective in stabilizing the membrane potential of erythrocyte membrane. The scavenging activity *in vitro* also proves that ADJ6 is effective against free radicals. **Conclusion:** Though exact mechanism hitherto unknown, it is evident that ADJ6 stabilizes erythrocyte membrane activity. Further studies will be conducted in order to prove the mechanism of action of ADJ6 against of free radicals.

Key words: Polyherbal formulation; ADJ6; Antioxidant; Erythrocyte ghost membrane; Na^+ K^+ ATPase.

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INTRODUCTION

Type II Diabetes Mellitus (T2DM) is currently classified as a metabolic disorder that precedes or leads to several other abnormalities such as hypertension, obesity, dyslipidaemia which increases the risk factors associated with cardiovascular, kidney and ophthalmic disorders.[1] It has been reported that T2DM has considerable effect on the rheology of red blood cells. T2DM also induces considerable alterations in the lipid composition of the RBC membranes resulting in erythrocyte deformity that may play a vital role in altering the blood rheological pattern resulting in atherosclerotic lesions.[2]

In Diabetes Mellitus, there is also a decreased activity of sodium potassium ATPase (Na^+ K^+ ATPase) along with glycation of proteins & oxidative stress on erythrocyte membrane.[3] Also free radicals such as H_2O_2 are prone for attacking the membrane composure increasing the incidence of lipid peroxidation.[4] It has also been reported that Na^+ K^+ ATPase activity is affected when lipid composition of the erythrocyte membrane is altered. Thus RBC Na^+ K^+ ATPase also plays a vital role in the regulation of intra & extra cellular homeostasis. Alteration of which is linked with several other complications such as hypertension, diabetes mellitus, nephropathy, peripheral neuropathy and microangiopathy.[5] Thus it is evident that Na^+ K^+ ATPase plays an inevitable role in the advent of many complications. Hitherto, many plants and their products have been tested against Type II diabetes. However many have not been proven effective in management of the diabetes. The present study aims to assess the potential of the polyherbal formulation ADJ6 against oxidative stress (i.e.) H_2O_2 induced oxidative damage via erythrocyte ghost membrane lipid peroxidation and its effect on ouabain controlled RBC Na^+ K^+ ATPase activity. The polyherbal formulation had already been reported by us for its inhibitory activity against α-amylase & α-glucosidase and also been studied for their phytochemical constituents by us previously.[6]

MATERIALS AND METHODS

Materials

Chemicals such as DPPH, ferrozine, HEPES, Ouabain, Tris-ATP, ascorbic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Ferric Chloride, EDTA, Thiobarbituric acid (TBA), trichloroacetic acid (TCA) were purchased from SRL Pvt. Ltd. (Mumbai, India). Hydrogen Peroxide was purchased from Qualigens Fine Chemical Pvt. Ltd. (Mumbai, India). All other reagents and chemicals were of analytical grade and procured locally in Chennai, India.

Preparation of ADJ6 extract

The plants (*Momordica charantia, Psidium guajava, Phyllanthus emblica, Trigonella foenum-graecum, Syzygium cumini* and *Gymnema sylvestre*) were collected from the medicinal farm of Frontier Mediville (Elavur, Gummidipoondi, India) and were submitted to the Plant Anatomy Research Centre (Tamil Nadu, India) for authentication (provided as Additional File 1).[6] The individual plants were minced using a mixer and freeze dried to prevent the loss of bioactive components. ADJ6 aqueous extract was prepared mixing them proportionally, suspending the components in water for 24 hrs and then filtered through Whatman No.1 filter paper. The Ayurvedic methods of preparing herbal formulations are primarily aqueous in order to denaturing the vital components.[6] The aqueous extract was used for antioxidant studies and studies involving erythrocytes.

DPPH Radical Scavenging Assay

0.2 mg/ml of ADJ6 aqueous extract was used for diphenyl-picrylhydrazyl (DPPH) radical assay, with some modifications[7] and a final concen-

tration range of 20-100 µg/ml was used for the assay. The sample was made up to 1 ml with 95% methanol followed by which 1 ml of 0.2 mM DPPH was added and incubated in dark for 30 min. The purple colour developed was read spectrophotometrically at 515 nm (Shimadzu UV-VIS 1800 spectrophotometer; Shimadzu Corporation, Kyoto, Japan). Ascorbic acid (20 µg-100 µg/ml) was used as standard and 95% methanol alone was used as blank. Reaction mixture with 95% methanol and DPPH alone was used as control. The % Inhibition was calculated using the formula:

$$\% I = (C - E) / C$$

Whereas % I=% Inhibition; C=Absorbance of control; E=Absorbance of extract

Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

The experiment was performed with some modifications.[8] Sample concentration range of 20 µg-100 µg/ml was used for the assay. 0.5 ml of 40 mM H_2O_2 (prepared in 50 mM phosphate, pH 7.4) was added to the sample and made up to 3 ml with phosphate buffer (50 mM, pH 7.4). The tubes were incubated in dark for 10 min. The absorbance was read in UV range at 230 nm. 40 mM H_2O_2 (in Phosphate buffer, 7.4) was used as control and ascorbic acid (20 µg-100 µg/ml) was used as standard and phosphate buffer alone was used as blank.

Metal Chelating Activity

The samples were taken in a concentration range of 20 µg-100 µg/ml and assay was performed with modifications.[9] 0.1 ml of 2 mM Ferric Chloride and 0.2 ml of 5 mM ferrozine was added to the sample and then made up to 1 ml with double distilled water. The reaction mixture was incubated for 10 min at room temperature. The red colour developed was read spectrophotometrically at 562 nm. EDTA (20 µg-100 µg/ml) was used standard. Tubes without sample or standard was used as control and double distilled water alone served as blank.

Collection of Blood Sample

The study was approved by Institutional Human Ethics Committee of Frontier Lifeline Hospital, Chennai (FLL/IEC/01/2014) and all the procedures were conducted in accordance to the regulatory guidelines prescribed by Ethical Guidance of Biomedical Research on Human Participants, ICMR, 2006. Whole blood was collected from healthy donors (n=5; 3 males & 2 females) using venipuncture method and was placed in EDTA coated tubes. The donors were non-obese, non-smokers, non-consumers of alcohol, normotensive and free of any other treatment.

RBC Membrane Stabilization Assay

Membrane Stabilization Assay was performed using Human erythrocytes and Alsever's solution with some modifications.[10] Blood was mixed with equal volume of Alsever's solution and centrifuged at 3000 rpm. The packed cell volume (PCV) was separated and washed with isosaline solution. The PCV was made up to 10% v/v suspension with isosaline. ADJ6 extract and ascorbic acid (positive control) were taken in a concentration range of 75, 100, 200, 300, 400, 500 µg/ml and were added to PCV in individual tubes. Following which 1 ml of 0.1 M phosphate buffer (pH 7.4) and 2 ml of hyposaline were added. The assay mixture was incubated at 37°C for 30 min and then centrifuged at 3000 rpm. The supernatant was discarded and the haemoglobin content was estimated using spectrophotometer at 560 nm (Shimadzu UV-VIS 1800 spectrophotometer; Shimadzu Corporation, Kyoto, Japan). Assay mixture with hyposaline and water served as control and blank respectively.

Isolation of Erythrocyte Ghost Membrane

Erythrocyte Ghost Membrane (EGM) was isolated[11] with slight modifications. 2 ml of blood sample was washed with ice cold Buffer 1 (140 mM sodium chloride & 5 mM disodium hydrogen phosphate, pH 8.0) for 4-5 times. The washed erythrocytes was then lysed with 40 volumes of ice cold Buffer 2 (5 mM disodium hydrogen phosphate, pH 8.0), incubated for 7 min on ice and centrifuged at 12000 rpm for 25 min at 4°C. This step was repeated several times until the haemoglobin content was removed from the membrane pellet. Then the pellet was washed with ice cold Buffer 3 (150 mM sodium chloride & 20 mM HEPES, pH 7.4). The procedure was repeated several times until the ghost membrane pellet turn pale in colour. Further the ghosts were subject to 10 freeze thaw cycles followed by which it was incubated for 2 h at 37°C. The ghost membrane was further used for lipid peroxidation studies and to assess the activity of sodium potassium ATPase immediately to achieve better results.

Activity of Sodium Potassium ATPase

The activity of Na^+ K^+ ATPase was determined with modifications.[12] 0.2 ml of ghost membrane with 1 ml of reaction mixture containing 3 mM Tris-ATP, 14 mM potassium chloride, 140 mM sodium chloride, 5 mM magnesium chloride, 1 mM EDTA and 50 mM Tris-HCl, pH 7.4 for 15 min in the presence or absence of Na^+ K^+ ATPase inhibitor, Ouabain (0.5 mM). The reaction mixture was incubated with ascorbic acid or ADJ6 in a concentration range of 20, 40, 60, 80, 100 µg/ml, for 30 min and then the reaction was stopped by adding 50 µl TCA. The inorganic phosphorous released was measured to determine the ATPase enzyme activity.[13] Na^+ K^+ ATPase activity was measured by the difference between the presence and absence of ouabain-sensitive Na^+ K^+ ATPase activity and expressed as µmolPi/mg prot/min.

Lipid Peroxidation of Erythrocyte ghost membrane

Lipid peroxidation in EGM was determined by measuring thiobarbituric acid reactive substances (TBARS) and was reported in terms of malondialdehyde generated with modifications.[14] 0.2 ml of ghost membrane was treated with 50 µl of 40 mM H_2O_2 and incubated for 30 min at 37°C. Followed by which ADJ6 and ascorbic acid were added to their respective tubes in a concentration range of 20, 40, 60, 80 & 100 µg/ml and then incubated at 37°C for 1 hr. Tubes without ADJ6/ghost membrane were used as blank while tubes containing all the components except ADJ6 or ascorbic acid were used as 100% oxidation control. 500 µl of 70% ethanol was added to arrest the reaction. To the reaction mixture, 1% TBA in sodium hydroxide and glacial acetic acid were added and was boiled in water for 15 min. The pink colour developed was spectrophotometrically measured at 532 nm. The result was expressed in terms of Malondialdehye liberated. The protein content of the erythrocyte ghost membrane was measured with some modifications.[15] Bovine Serum Albumin (BSA) was used as the standard.

Statistical Analysis

All the experiments were performed in three different sets with each set duplicated. The data were expressed as the Mean ± The Standard Error Mean (SEM).

RESULTS & DISCUSSION

In vitro Antioxidant Activity

The inhibitory activity of the polyherbal formulation ADJ6 (Figure 1a) against DPPH radical (IC_{50}=41.88 ± 1.467 µg/ml) was better when compared to the scavenging activity of ascorbic acid (IC_{50}=60.7 ± 0.166 µg/ml). The hydrogen peroxide scavenging activity of ADJ6 (Figure 1b) (IC_{50}=67.08 ± 2.767 µg/ml) was almost equivalent when compared to ascorbic acid (IC_{50}=65.78 ± 3.548 µg/ml). The metal chelating activity of ADJ6 (Figure 1c) (IC_{50}=49.46 ± 4.545 µg/ml) was much better when compared to that of EDTA (IC_{50}=62.95 ± 2.92 µg/ml).

Figure A: Antioxidant Analysis of ADJ6

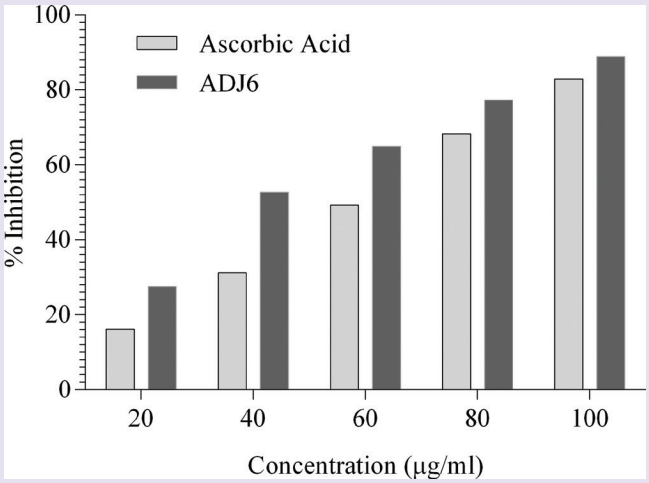

Figure 1a: DPPH radical scavenging activity (n=3)
IC_{50}: ADJ6=41.88 ± 1.467 µg/ml; Ascorbic acid=60.7 ± 0.166 µg/ml.

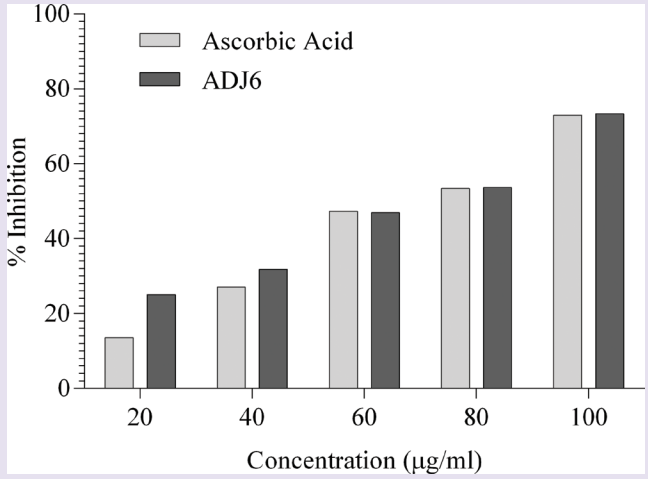

Figure 1b: Hydrogen Peroxide Radical Scavenging Activity (n=3)
IC_{50}: ADJ6=67.08 ± 2.767 µg/ml; Ascorbic acid=65.78 ± 3.548 µg/ml.

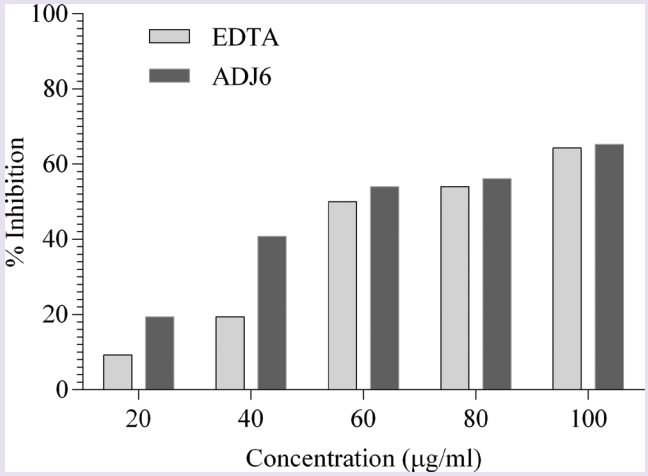

Figure 1c: Metal chelating activity (n=3)
IC_{50}: ADJ6=49.46 ± 4.545 µg/ml; EDTA=62.95 ± 2.92 µg/ml.

RBC Membrane Stabilization Activity

In the present study, (Figure 2a) RBC haemolysis was induced by hypotonicity and the membrane stabilization potential of ADJ6 was determined using six different concentrations. ADJ6 depicted effective protection against the hypotonicity induced haemolysis (IC_{50}=90.83 ± 1.905 µg/ml) with increase in concentration in comparison with standard ascorbic acid (IC_{50}=91.06 ± 2.018 µg/ml), in a dose dependent manner.

Lipid Peroxidation

In the present study (Figure 2b & Figure 2c), initially when compared to ascorbic acid (IC_{50}=87.933 ± 0.973 µg/ml, 0.401 ± 0.011 µM of malondialdehyde), the inhibition of lipid peroxidation by ADJ6 reduced (IC_{50}=84.5 ± 2.613 µg/ml, 0.386 ± 0.004 µM of malondialdehyde). This shows that ADJ6 minimizes the production of malondialdehyde produced by lipid peroxidation thus reducing the adverse effects incurred by the erythrocyte membrane.

Sodium Potassium ATPase Activity

The present study shows treatment with ADJ6 elevated the activity of Na^+ K^+ ATPase activity (Figure 2d) in comparison with standard ascorbic acid suggesting maintenance of membrane homeostasis mechanism by ADJ6.

DISCUSSION

Free radicals are commonly recognized as atoms having one or more unpaired electrons that cause destruction to macromolecules, in turn creating a chain reaction damaging the cellular integrity of the living system leading to oxidative stress and related disorders.[16] Mean corpuscular Fragility (MCF) is known to be a technique which enumerates the concentration of saline causing 50% haemolysis in erythrocytes. This elucidates how plants can maintain membrane stability against hypotonic solution induced stress.[17] In the present study, we observe that

Figure B: Effect pf ADJ6 on Human erythrocytes, *in vitro*

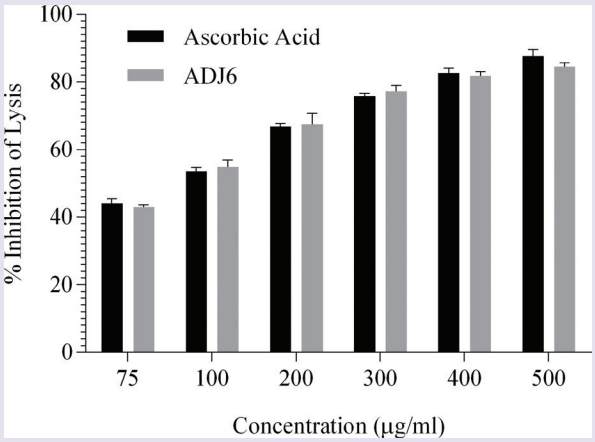

Figure 2a: RBC Membrane Stabilization Assay (n=3)
IC_{50}: ADJ6=90.83 ± 1.905 µg/ml; Ascorbic acid=91.06 ± 2.018 µg/ml.

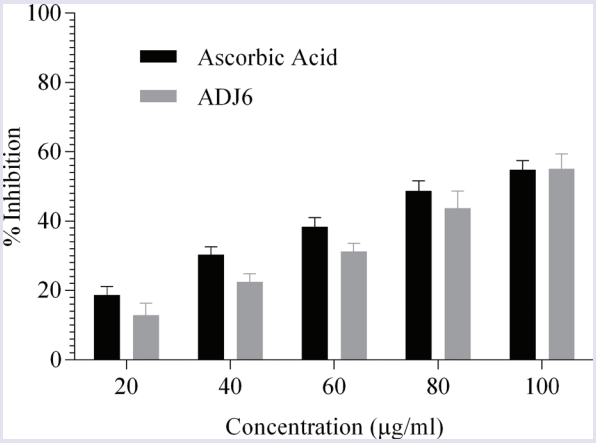

Figure 2b: Inhibition of Lipid Peroxidation (n=3)
IC_{50}: ADJ6=84.5 ± 2.163 µg/ml; Ascorbic acid=87.933 ± 0.973 µg/ml.

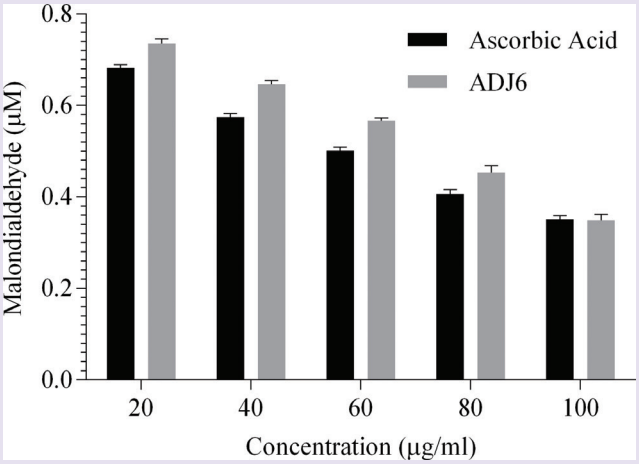

Figure 2c: Estimation of Malondialdehye (n=3)
Ascorbic acid=0.401 ± 0.011 µM of malondialdehye; ADJ6=0.386 ± 0.004 µM of malondialdehye.

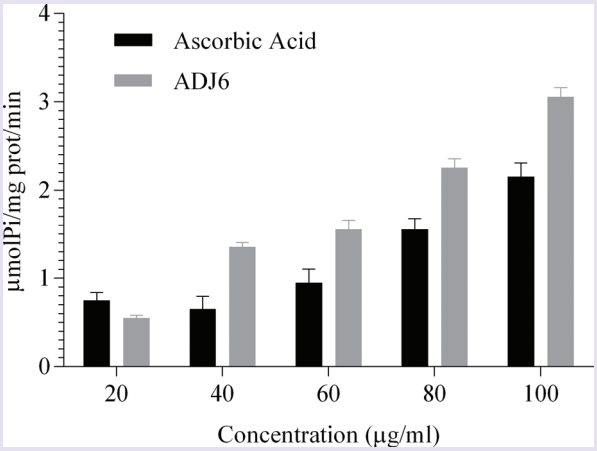

Figure 2d: Na^+K^+ ATPase Assay (n=3)
Activity of ADJ6 & Ascorbic acid against ouabain induced Na^+K^+ ATPase enzyme.

ADJ6 effectively prevent lysis of RBC by stabilizing the membrane at par with the standard drug ascorbic acid. Previous studies suggest that H_2O_2 induces deformability of erythrocytes[18] and increased oxygen radicals can initiate lipid peroxidation and increase MDA accumulation.[19] Studies have also revealed that oxidative stress mediated lipid peroxidation disrupts structure, alters integrity, fluidity, permeability, functions of biomembranes and produces toxic intermediates. Lipid peroxidation of cellular membranes also increases polarity, forms oligomers, alters molecular mobility and resistance to thermo denaturation.[20] It has been reported that decrease in the erythrocyte membrane Na^+ K^+ ATPase can disturb the cellular cation homeostasis. Also ROS^- induced lipid peroxidation alters the Na^+ K^+ ATPase activity leading to cellular lysis.[3] Also the amount of free radical present in the body accounts for the decrease or inhibition in the activity of Na^+ K^+ ATPase[21] Studies have also revealed that the complications of major diseases such as diabetes mellitus arise from oxidative stress and subsequent generation of reactive oxygen species leading to its pathogenesis and cellular damaging processes. As discussed earlier, it is evident that free radicals initiate lipid peroxidation thus damaging cellular processes[21] It has been denoted that there is a decrease in Na^+ K^+ ATPase upon oxidation stress of erythrocyte membrane in diabetic patients and decrease in the enzyme activity is correlated with the duration of diabetes.

This study on ADJ6 proves that it has the ability to scavenge free radicals, stabilizes RBC membrane and inhibits H_2O_2 induced lipid peroxidation in a dose-dependent manner. ADJ6 also increases Na^+ K^+ ATPase activity considerably. Though the mechanism underlying the activity of ADJ6 is unknown, based upon the preliminary studies and in the view of the available literature we suggest that the plants used in ADJ6 are rich in antioxidants and have the potential to fight against free radical induced damage. Thus ADJ6 can be considered as a viable prospect in the management of oxidative stress and diabetes. The present study is a pilot study consisting of limited number of samples. Further

insights should be explored in order to elucidate the ability of ADJ6 to combat free radicals, minimize free radical induced damages and can also maintain cellular homeostasis.

CONCLUSION

The present study demonstrated the free radical scavenging and membrane stabilizing activity of ADJ6. The study also established that ADJ6 prevents free radical induced lipid peroxidation of RBC membrane and also stabilizes the activity of ATPase enzyme. Thus, ADJ6, rich in antioxidants can be potentially used to target free radicals minimizing the adverse effects arising due to disorders. Further investigations will be carried out to elucidate the exact mechanism involving in the protective effect against free radicals and against T2DM.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

T2DM: Type II Diabetes Mellitus/Type 2 Diabetes Mellitus; **Na^+K^+ ATPase:** Sodium Potassium ATPase enzyme; **H_2O_2:** Hydrogen Peroxide; **RBC:** Red blood cells; **DPPH:** Diphenyl-picrylhydrazyl; **HEPES:** (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); **Tris-ATP:** Tris Adenosine Triphosphate; **TBA:** Thio barbituric acid; **TCA:** Trichloracetic acid; **UV-VIS:** Ultra violet & visible range; **ICMR:** Indian Council for Medical Research; **PCV:** Packed Cell Volume; **EGM:** Erythrocyte ghost membrane; **Tris-HCl:** Tris hydrochloride; TBARS: Thiobarbituric acid reactive substances; BSA: Bovine Serum Albumin; **SEM:** Standard Error Mean; **IC_{50}:** Inhibitory concentration; **MCF:** Mean Corpuscular Fragility; **EC_{50}:** Effective Concentration; **MDA:** Malondialdehyde.

REFERENCES

1. Edelman SV. Type II diabetes mellitus. Advances in Internal Medicine. 1998;43:449-500.
2. Prisco D, Paniccia R, Coppo M, Vanni D, Rogasi P, Tramontana M *et al*. Red blood cell lipid alterations in type II diabetes mellitus. Thrombosis Research. 1989;54(6):751-8.
3. Kumar R, Kumar AN, Ahmed S. Changes in Erythrocyte Membrane in Type-2 Diabetes Mellitus with and without Dyslipidemia. J Diabetes Metab. 2011;2(6):141.
4. Uzel N, Sivas A, Uysal M, Ã–z H. Erythrocyte Lipid Peroxidation and Glutathione Peroxidase Activities in Patients with Diabetes Mellitus. Hormone and Metabolic Research. 1987;19(02):89-90.
5. Totan A, Greabu M. Effect of chronic hyperglycemia and vanadate treatment on erythrocyte Na^+/K-ATPase and Mg-ATPase in streptozotocin diabetic rats. Acta poloniae pharmaceutica. 2012;59(4):307-11.
6. Duraiswamy A, Shanmugasundaram D, Sasikumar C, Cherian S, Cherian K. Development of an antidiabetic formulation (ADJ6) and its inhibitory activity against α-amylase and α-glucosidase. Journal of Traditional and Complementary Medicine. 2015 (http://dx.doi.org/10.1016/j.jtcme.2014.12.006).
7. Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995;28(1):25-30.
8. Ebrahimzadeh M, Nabavi S, Nabavi S, Bekhradnia A. Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *Angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*. Pakistan Journal of Pharmaceutical Sciences. 2009;23(1):29-34.
9. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from Osbeckia parvifolia Arn.-An *in vitro* approach. Journal of King Saud University-Science. 2014;26(4):267-75.
10. Saleem T, Azeem A, Dilip C, Sankar C, Prasanth N, Duraisami R. Anti-inflammatory activity of the leaf extacts of Gendarussa vulgaris Nees. Asian Pacific Journal of Tropical Biomedicine. 2011;1(2):147-9.
11. Mikelj M, Praper T, Demi ÄR, Hodnik V, Turk T, Anderluh G. Electroformation of giant unilamellar vesicles from erythrocyte membranes under low-salt conditions. Analytical Biochemistry. 2013;435(2):174-80.
12. Reinila M, MacDonald E, Salem N, Linnoila M, Trams E. Standardized method for the determination of human erythrocyte membrane adenosine triphosphatases. Analytical Biochemistry. 1982;124(1):19-26.
13. Jayaraman J. Laboratory manual in biochemistry. New Delhi: New Age International;1981:13.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979;95(2):351-8.
15. Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. Advance in Internal Medicine. 1951;193(1):265-75.
16. Chinaka ON, Onyeka VA, Isaac UA. Membrane Stability of Red Blood Cells in Diabetic Mice Treated with D-3-O-Methylchiroinositol. AJPS. 2014;2(1):24-6.
17. Srour M, Bilto Y, Juma M, Irhimeh M. Exposure of human erythrocytes to oxygen radicals causes loss of deformability, increased osmotic fragility, lipid peroxidation and protein degradation. Clinical Hemorheology and Microcirculation. 2000;23(1):13-21.
18. Jain S, Palmer M. The Effect of Oxygen Radicals Metabolites and Vitamin E on Glycosylation of Proteins. Free Radical Biology and Medicine. 1997;22(4):593-6.
19. Pandey K, Rizvi S. Markers of Oxidative Stress in Erythrocytes and Plasma During Aging in Humans. Oxidative Medicine and Cellular Longevity. 2010;3(1):2-12.
20. Gurel A, Armutcu F, Damatoglu S, Unalacak M, Demircan N. Evaluation of erythrocyte Na^+, K^+ -ATPase and Superoxide Dismutase activities and malondialdehyde level alteration in coal miners. European Journal of General Medicine. 2004;1(4):22-8.
21. Davi G, Falco A, Patrono C. Lipid Peroxidation in Diabetes Mellitus. Antioxidants & Redox Signaling. 2005;7(1-2):256-68.

SUMMARY

- ADJ6 was effective against *in vitro* free radicals.
- Minimized the hypotonicity induced RBC haemolysis and also aided membrane stabilization.
- Effectively reduces the H_2O_2 induced lipid peroxidation of RBC membrane.
- Stabilizes the cationic membrane enzyme Na^+ K^+ ATPase.
- ADJ6 stabilizes RBC membrane and scavenges the free radicals in a dose dependent approach.

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Dr. Sanjay M Cherian: Consultant cardiac surgeon at Frontier Lifeline Hospital, Chennai. He has been involved in various clinical research projects involving cardio vascular diseases and stem cell therapy. Currently one of his interests is developing herbal medication for treating diabetes.

Dr. Kotturathu Mammen Cherian is the Chief & CEO of Frontier Lifeline & Dr. K. M. Cherian Heart Foundation. He is a pioneer in paediatric cardiac treatments & heart transplant surgeries. His vision is for the upliftment of Ancient Indian Medicine & to explore the potential of herbal medicine for various therapies including cardiovascular diseases & diabetes.