Original Research Article **pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

FREE RADICAL SCAVENGING EFFECT OF ADENIUM OBESUM IN STREPTOZOTOCIN CHALLENGED DIABETIC RATS.

SIREESHA KALVA[1]* AND RAGHUNANDAN. N[2]

[1]Department of pharmacology, Sri Venkateshwara College of pharmacy and research centre, Madhapur, Hyderabad, Telangana, India.
[2]Department of pharmaceutical sciences, Balaji Institute of pharmaceutical sciences, Narsampet, Warangal, Telangana, India.

ABSTRACT

The present study was aimed to investigate the *invitro and invivo* antioxidant activities of two extracts i.e, methanolic extract and ethylacetate extract of *Adenium obesum* (MEAO,EAEAO)flowers which belong to family apocyanacea. The antioxidant activities of the two extracts had been evaluated by using standard invitro and invivo models in streptozotocin induced diabetic rats. The invitro antioxidant studies were determined by the percentage inhibition values estimated for DPPH(2,2-diphenyl-1-picrylhydrazyl) and Nitric oxide assays.The invivo antioxidant study was determined by evaluating enzymatic antioxidants such as Superoxide dismutase (SOD), Lipidperoxidase(LPO) and Catalase(CAT) in hepatic tissues of STZ induced diabetic rats.The results showed a significant percent inhibition in DPPH and NO assay when compared to diabetic control group.Ascorbic acid is taken as a standard antioxidant. The drug treated groups has shown an increase in the SOD, CAT and decreased Melondialdehyde levels as well significantly. Based on this study it was concluded that the methanolic and ethylacetate extracts of dried flowers of adenium obesum showed potent invivo and invitro antioxidant activity in streptozotocin challenged diabetic rat models.

KEYWORDS: ***Melondialdehyde, nitric oxide ,DPPH, streptozotocin.***

SIREESHA KALVA*

Department of pharmacology, Sri Venkateshwara College of pharmacy and research centre, Madhapur, Hyderabad, Telangana, India.

***Corresponding Author**

Received on: 15-04-2017
Revised and Accepted on: 22-05-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.3.p34-41

INTRODUCTION

The effect of free radicals on human beings are closely related to toxicity, disease and ageing. [1]Most living species have an efficient defence system to protect themselves against oxidative stress induced by reactive oxygen species(ROS).[2]There is increasing evidence that complication related to diabetes are associated with oxidative stress induced by generation of free radicals.[3]Over production of various forms of activated species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and non free radical species is considered to be the main contributors to oxidative stress. Epidemiological data as well as *in vitro* studies, strongly suggest that plants containing phytochemicals with antioxidant potential have strong protective effects against major disease risks caused due to oxidative stress.[4]In diabetes oxidative stress has been found to be mainly due to an increased prodction of oxygen free radials and sharp reduction of antioxidant defences. [5]Hence compounds with antioxidative property would be useful antidiabetic agents. [6]Streptozotocin induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage.[7] Diabetes and experimental animal models exhibit oxidative stress due to persistant and chronic hyperglycaemia, which there by depletes the activity of antioxidant defence system and thus promotes de novo free radical generation.[8] Natural products and herbal medicines are good source of new therapeutic agents and for the development of complementary and alternative medicines over traditional drug regimens.[9]Anthocyanins are representative of plant pigments widely distributed in colored fruits and flowers. Because anthocyanins are widely consumed, finding out additional biological activities related tothese compounds would be of great interest .[10]Ascorbic acid is a redox catalyst which can reduce, and there by neutralise reactive oxygen speices such as hydrogen peroxidase. [11]In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase a function that is particularly important in stress resistance in plants. Ascorbic acid is present in high levels in all plants and can reach concentration of 20 millimoles in chloroplast.[12]In the last few years,there has been an exponential development in the field of herbal medicine and their phytomedicines are gradually gaining popularity both in developed and developing countries due to their origin in nature, more potent in treatment of health problems and less side effects as compared to marketed drugs. [13]Between 10000 and 53000 species of plant are utilised in traditional medicine and use of plants in medicine now an impotant and ubiquitous culture trait.[14] *Adenium obesum* commonly known as desert rose belonging to the family apocyanaceae, is native from Africa, Omen, Saudi Arabia and Yemen as wild plant.[15]*Adenium obesum* is an important medicinal plant which shows a wide range of biological activities like anticancer,anti viral, antibacterial activities.[16] *Adenum obesium* flower extracts are rich source of anthocyanins .Therefore it seemed worthwhile to assess the antioxidant potential of methonolic and ethylacetate extracts of *Adenum* by standard i*nvivo* and i*nvitro* methods.

MATERIALS AND METHODS

The flowers of Adenum obesium were collected from Tirumala Hills, Tirupathi, Andhrapradesh during the month of July- Aug 2015. The authentication was done by S.V University, Botony department, Tirupathi bearing a voucher no.1242.

Drugs and chemicals

Streptozotocin was procured from Sisco Research Laboratories pvt ltd. Mumbai-93,India. Batch No: T-835796, Metformin was a gift sample from Ranbaxy pvt ltd, Mouhali, Punjab, India. Methanol was procured from SDFCL, India. All other chemicals were procured from Ramkem,India. The all chemicals used were of analytical grade.

Preparation of Extract

The flowers obtained were dried under shade. The dried flowers were crushed, placed in a closed vessel. To the powder required quantities of methanol as well ethylacetate were added separately in two different containers and allowed to stand for 7 days with occasionally shaking. The liquid was strained off, solid residue was pressed, clarified by filteration and then subjected to evoparation.

Preliminary phytochemical Testing

The extracts were subjected to preliminary phytochemical testing to detect for the prescence of different chemical groups of compounds. Air dried and powdered plant materials were screened for the presence of saponins,tanins, alkaloids , glycosides, anthocyanins, carbohydrates,reducing sugars,starch, protein and aminoacids .

Animals

Animal protocol was approved by Institutional animal ethical committee (IAEC) for purpose of control and supervision of experimentation on animals (CPCSEA) through its reference no. IAEC/SVCP/2011/007- dated 26/7/11. Male wistar rats each weiging 180-250 gm were obtained from National Institute Of Nutrition (NIN), Hyderabad. The animals were housed with free acess to food and water for atleast one week in air conditioned room (25°c) for 12 hours for light dark cycle adaptation prior to the experiment. They were fed with standard diet (Hindhusthan lever pvt.ltd.) and water *ad libitum*.

Experimental design

Group 1 - Control
Group 2 - Diabetic control
Group 3 - Diabetes+METFORMIN (50mg/kg)(p.o)
Group 4 - N+MEAO (100mg/kg) (p.o)
Group 5 - N+MEAO (200mg/kg) (p.o)
Group 6 - N+MEAO (300mg/kg) (p.o)
Group 7 - D+MEAO (100mg/kg) (p.o)
Group 8 - D+MEAO (200mg/kg) (p.o)
Group 9 - D+MEAO (300mg/kg) (p.o)
Group 10 - N+EAEAO (100mg/kg) (p.o)
Group 11 - N+EAEAO (200mg/kg)(p.o)
Group 12 - N+EAEAO (300mg/kg) (p.o)
Group 13 - D+EAEAO (100mg/kg) (p.o)
Group 14 - D+EAEAO (200mg/kg) (p.o)
Group 15 - D+EAEAO (300mg/kg) (p.o)

Rats were divided randomly into fifteen groups of six animals each and treated for 14 days as follows. Group 1 animals served as normal control group received normal saline in a dose of 10ml/kg. Group II served as diabetic control (Streptozotocin induced diabetic rats, STZ dose of 35mg/kg,p.o) was given physiological saline solution. Group III include diabetic rats administered with Metformin (50mg /kg body weight). Group IV ,V,VI include normal rats treated with Methanolic extract of Adenum obisium at 100, 200,300 mg/kgb.w/day. Group VII,VIII,IX include diabetic rats treated with Methanolic extract of Adenum obisium (MEAO) at 100, 200,300 mg/kgb.w/day. Group X,XI.XII are normal rats treated with Ethylacetate extract of Adenum obisium (EAEAO) at 100, 200,300 mg/kgb.w/day Groups XIII.XIV,XV include diabetic rats treated with ethylacetateextract of *Adenum obisium* at 100,200,300 mg/kgb.w/ day. After 14 days the liver is dissected out and estimated for antioxidant enzymes.

Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenised in chilled Tris-HCl buffer (0.025M, p^H 7.4) using homogenizer. The homogenate obtained was centrifuged at 5000rpm for 10 mins,supernatant was collected and used for various *in vivo* antioxidant assays.

Acute toxicity studies

The acute toxicity study for MEAO and EAEAO was done according to the OECD guidelines N0. 423. The extracts did not show any noticeable signs of toxicity when given in doses upto 2000mg/kg by an oral route. Hence, for further studies three doses 100, 200 and 300mg/kg body weightwere selected.

Streptozotocin induced Diabetes

A freshly prepared solution of Streptozotocin STZ (35mg/kg in 0.1M citrate buffer,p^H.5) was injected intraperitoneally to over night fasted rats. The rats showed hyperglycaemia with in 48 h of STZ administration. The rats having Fasting Blood Glucose (FPG)of 250 mg/dl or above were selected for the study.

Invivoantioxidant studies

Lipid peroxidation is based on the reaction of Melondialdehyde with thio barbituric acid to form Thiobarbituric acid reactive substances (TBARS), which has a pink colour with aborbtion maxima at 540 nm. Catalase (CAT) is determined by Aebi method and super oxide dismutase (SOD) activity was determined colorimetrically by the method of Kono.[17,18.]

Invitro studies

The free radical scavenging activity of *Adenum obesium* was measured by 1,1- diphenyl-2-picryl-hydrazil (DPPH) using the method of Sreejayan method.[19] 1ml of different concentrations 50,100,200,300,400 and 500ug/ml of the test substance and standard were taken in different testtubes. To this, add 3ml of methanolic solution of DPPH and incubated at 37C for 20 min. The absorbance was measured at 517nm on a spectrophotometer (UV-pectrophotometer). Ascorbic acid is used as standardantioxidant agent.The concentration of the test extracts required to decrease the initial concentratin by 50% (IC50) was calculated.

Scavenging of Nitric Oxide Radical

At physiological p^H sodium nitropruside in aqueous solution spontaneously generates nitric oxide, producing nitrite ions by interacting with oxygen which can be determined by Griess reagent.[20]In the present investigation,Nitrite ions reacting with Griess reagent forms a purple azo dye. In the prescence of test sample, the number of nitrite formation decreases. The degree of decreased intensity of purple azo dye formation will influence the scavenging property of the sample, the absorbance was measured at 540nm. 0.5ml of exarcts at various concentrations 50,100,200,300,400 and 500 ug/ml were mixed with 2ml of 5mMsodium nitropruside in 0.5ml phosphate buffer saline (PH 7.4,1ml) and the mixture was incubated at 25°c for 150 min. From this mixture 0.5ml was taken and 1ml sulfannilic acid was added and incubated at room temperature for 5 min. Finally 1ml of NEDD was mixed and incubated at room temperature for 30 min. The absorbance was measured at 540 nm with a spectrophotometer. The experiment was performed in triplicates.

RESULTS

Acute toxicity studies

Acute oral toxicity studies revealed that MEAO and EAEAO was found to be safe up to a dose level of 2000mg/kg body weight p.o. in rats. There was no lethality (or) any toxic reactions were observed up to the end of the study period with LD_{50}>2000 mg/kg body weight.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of dried flowers of *Adenium obesum* were showed the prescence ofanthocyanins, glycoside, tannins, protiens in both the extracts.

Invivo anti oxidant study

In the present study STZ induced diabetic rats displayed significant ($p<0.001$) elevation of malondialdehyde MDA,a reliable marker for lipid peroxidation in the hepatic tissue homogenate when compared with normal group. Whilst, treatment wih MEAO (100,200,300 mg/kg) significantly reduced the MDA levels normally ($p <0.001$)shown in table. 1.The SOD and CAT levels were significantly ($p<0.001$) decreased in the liver of STZ induced dibetic rats. Oral administration of MEAO (300mg/kg) significantly restored the depleted ($p<0.001$)antioxidant levels to normal.MEAO (100mg/kg) doesn't per se any significant effects on the SOD and CAT. While groups treated with EAEAO p.o. (200 & 300mg/kg) also showed significant reduction ($p<0.001$) in the MDA levels where as the SOD and CAT levels were significantly raised in the EAEAO treated groups ($p<0.001$). The SOD and CAT levels were significantly ($p<0.001$) decreased in liver of STZ induced diabetic rats and the Malondialdehyde levels increased in the same group compared to the normal group. Both the extracts of *Adenum obesium*significantly increased the SOD and CAT levels, decreased the Malondialdehyde respectively in the drug treated groups. Fig.1&2

Table 1
Effect of MEAO and EAEAO on LPO, SOD, CAT on nomal and diabetic rats.

Groups	LPO	SOD	CAT
Normal	15.1± 1.44	37.67 ± 1.36	20.4 ± 1.79
Diabetes	47.6 ± 1.52	19 ± 0.77	6.6 ± 1.28
D+ Metformin	17.8 ± 1.56	48.25 0.51	11.93 ± 0.865
N+MEAO(100mg/kg)	14.25 ± 1.14	34.93± 1.20	20.43± 1.69
N+MEAO(200mg/kg)	17.80 ± 1.21	35.83 ± 1.39	19.73 ± 1.20
N+MEAO(300mg/kg)	17.54 ± 1.944	35.4 ± 2.67	22.3 ± 1.10
D+MEAO(100mg/kg)	39.77± 2.16[a]	21.69 ± 1.06[a]	6.1± 1.2[ab]
D+MEAO(200mg/kg)	22.64 ± 2.23[a,b]	29.32 ± 2.40[a,b]	9.15± 0.49[a,b]
D+MEAO(300mg/kg)	18.96±1.39[a,b]	42.05± 1.02[a,b]	13.40 ± 0.938[a,b]
N+EAEAO(100mg/kg)	15.05 ± 1.41	36.89 ± 1.15[a]	21.30 ± 1.59[a]
N+EAEAO(200mg/kg)	16.85 ± 0.77[a]	34.40 ± 1.16	22.60± 2.39
N+EAEAO(300mg/kg)	15.83± 1.41	34.13 ± 1.32	21.46± 2.01
D+ EAEAO(100mg/kg)	43.51 ± 1.38[a]	30.91± 0.850[a]	6.62± 1.13[ab]
D+EAEAO(200mg/kg)	21.21 ± 1.60[ab]	36.92± 1.29[ab]	12.28 ± 0.701[ab]
D+EAEAO(300mg/kg)	19.27± 0.785[ab]	47.15 ±1.59[ab]	12.65 ± 1.19[ab]

Values are repoted as Mean±SEM. a- Drug treated group compared to normal group (P<0.001); b- Drug treated group compared to diabetic group(P<0.001).

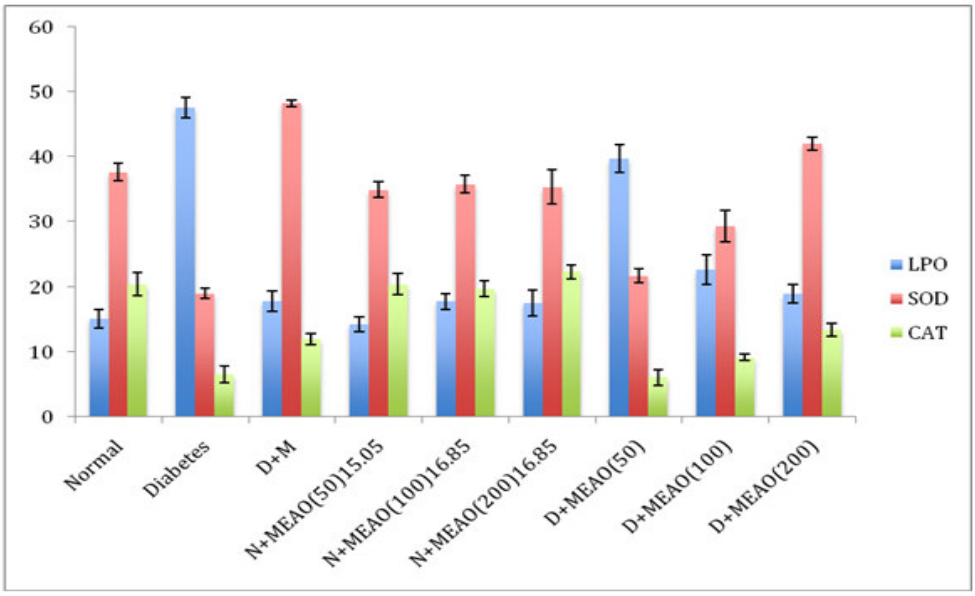

Figure 1
Effect of methanolic extract of Adenium obesum on Antioxidant enzymes in Diabetic rats.

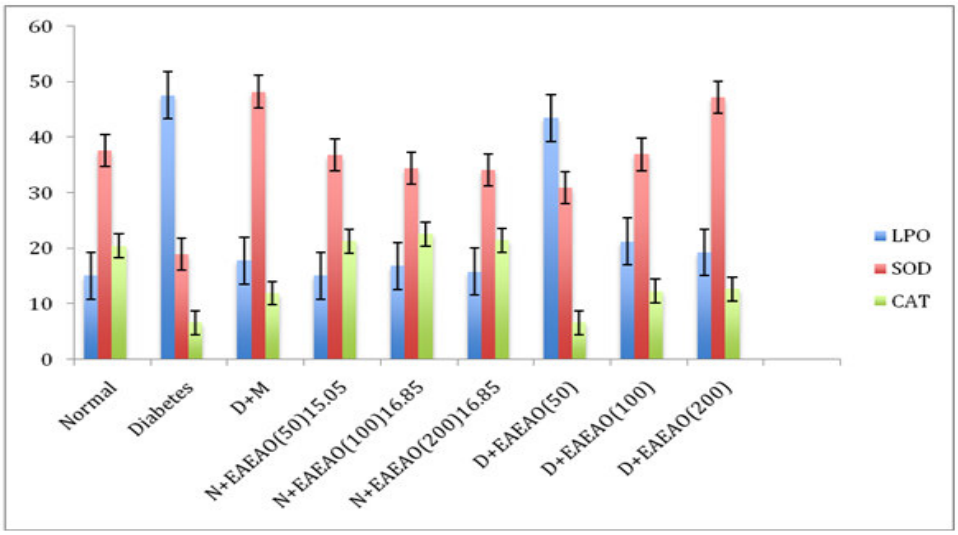

Figure 2
Effect of Ethylacetate extract of Adenum obesium on Antioxidant enzymes in Diabetic rats

Invitro antioxidant study

DPPH free radical scavenging activity.

In the free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts an elcetron or hydrogen radical to become stable molecule. The reduction capability of DPPH radical caused by antioxidants was determined by decreased in its absorbance at 517nm. *Adenum obesium* exhibited a comparable antioxidant activity with that of the standard ascorbic acid at varying concentrations tested (50,100,200,300,400,500ug/ml). There was a dose dependent increase in the percentge inhibition on atioxidant activity for all concentrations. (Table 2) The methanolic extract at a concentration 50ug/ml showed a percent inhibition of 10.05±0.29 and at 500ug/ml it was 80.61±0.56. The ethylacetate extract of *Adenum obesium*at a concentration of 50ug/ml showed 9.37±0.17 and at 500ug/ml 82.56±0.37. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. Ascorbic acid at a concentration of 50ug/ml exhibited a percentage inhibition of 6.14±0.17 and at 500ug/ml 91.78±0.37 (Table 2.). A graded increase in percentage of inhibition was observed with the increase in concentration of extracts. The IC 50 value of Ascorbic acid was found to be295ug/ml and the IC 50 value of sample extracts (MEAO & EAEAO) were found to be 290ug/ml and 255ug/ml respectively.

Table 2

DPPH radical scavenging activity of Methanolic extract (MEAO) and Ethylacetate extract (EAEAO) of Adenum obesium flower.

	50ug/ml	100 ug/ml	200 ug/ml	300 ug/ml	400ug/ml	500ug/ml
Ascorbic acid	6.14±0.17	13.75±0.20	32.06±0.30	51.13±0.44	67.86±0.20	91.78±0.40
MEAO	10.05±0.294	19.22±0.205	34.04±0.58	52.16±0.583	66.28±0.65	80.61±0.202
EAEAO	9.37±0.23	17.8±0.28	33.71±0.203	48.85±0.364	75.9±0.66	82.56±1.4

Scavenging of NO radical method

Both the extracts effectively reduced the generation of nitric oxide from sodium nitropruside. Scavenging of nitric oxide radical is based on the generation of nitric oxide . Sodium nitropruside in buffered saline, reacts with oxygen to produce nitrate ions that is measured by using Griess reagent . The absorbance is measured at 540nm. Table 3 shows % inhibition values at different concentrations. Ascorbic acid at a concentration of 50ug/ml exhibited a percentage inhibition of 10.02±0.15 and at 500ug/ml 93.98±0.37 The MEAO and EAEAO have shown signifcant ($p<0.001$) inhibition at a concentration of 500ug/ml 88.77±0.56 and 81.96±0.37 respectively.Fig. 4 shows the % inhibition values of nitric oxide assay of MEAO and EAEAO . The IC_{50} value of Ascorbic acid was found to be 255ug/ml, IC_{50} of MEAO and EAEAO are 290ug/ml and 285 ug/ml respectively. Fig.5 & 6 are the standard graphs of Ascorbic acid for DPPH and NO assay.

Table 3

NO radical scavenging activity of Methanolic extract (MEAO) and Ethylacetate extract (EAEAO) of Adenum obesium flower

	50ug/ml	100ug/ml	200ug/ml	300ug/ml	400ug/ml	500ug/ml
AscorbicAcid	10.02±0.15	18.82±0.25	30.48±0.60	53.48±0.408	73.08±0.18	93.98±0.37
MEAO	13.38±0.34	22.99±0.44	37.96±0.46	51.86±0.42	78.43±0.36	88.77±0.56
EAEAO	8.91±0.17	21.87±0.25	38.71±0.38	54.46±0.73	76.08±0.79	81.96±0.37

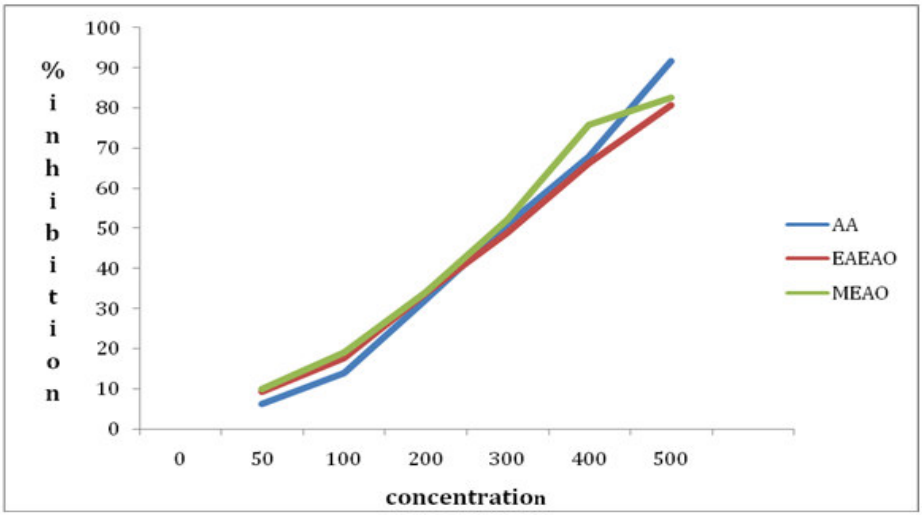

Figure 3

Effect of DPPH radical scavenging activity of MEAO and EAEAO of Adenium Obesum flower extracts

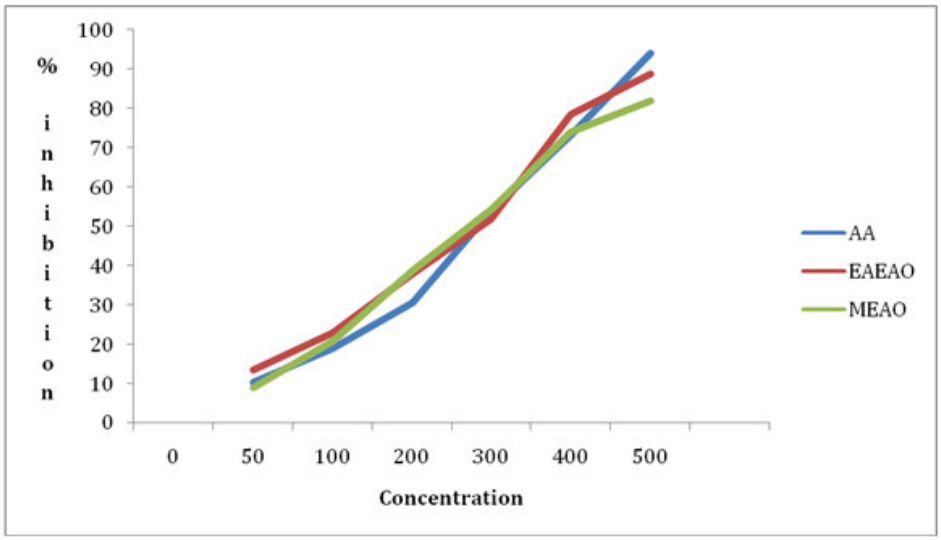

Figure 4
Effect of NO radical scavenging activity of MEAO and EAEAO of Adenium Obesum flower extracts

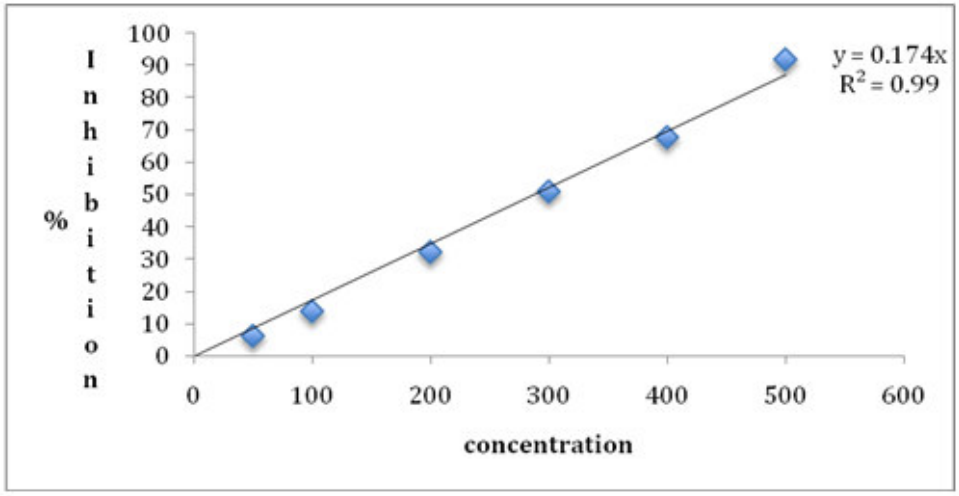

Figure 5
Standard graph of ascorbic acid –DPPH activity

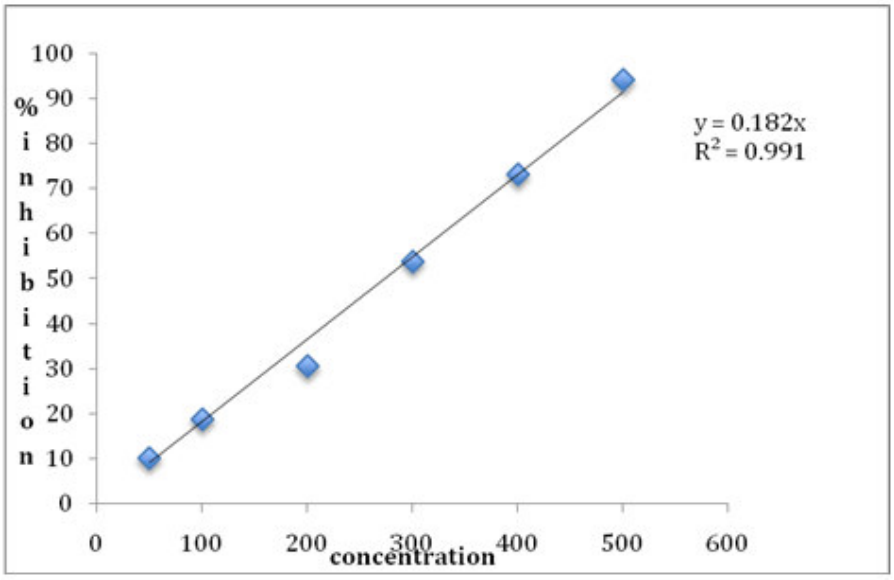

Figure 6
Standard graph of ascorbic acid- NO

DISCUSSION

In the biological system, free radicals are naturally generated in a constant manner during various metabolic reactions, where they can cause an extensive damage to the tissues generating complicated diseases such as diabetes, atherosclerosis, neurodegenerative diseases, cancer and Rheumatoid arthritis. In diabetes mellitus, this oxidative damage to the tissues proceeds to the appearance of various complications which maybe fatal. Streptozotocin (STZ) is a noxious chemical agent used to induce a state of hyperglycaemia in preclinical models. It induces diabetes due to rampant generation of reactive oxygen (ROS) that causes rapid ATP dephosphorylation which may lead to substrate availability for xanthine oxidase.So, these series of toxic eventslead generation of superoxide radicals,hydrogen peroxide, and hydroxyl radicals and causes oxidative pancreatic β cell damage.[21] In the present study,STZ significantly induced hyperglycaemia.In Diabetes, due to the generation of free radicals, there is tissue damage due to the formation of lipid peroxidase.[22] With the treatment of flower extract a significant decrease in the lipid peroxidation and improvement of anti-oxidant status were observed which may contribute to the prevention of diabetic complications.In this context, the free radical scavenging activity of MEAO and EAEAO might be due to the effect of bioactive principle anthocyanins which has also contributed for the effective radical scavenging property present in the flowers of *Adenum obesium.*DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Both the extracts showed dose dependent DPPH radical scavenging activity. The decrease in the absorbance of DPPH caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in scavenging of the radical by hydrogen donation.[23]It was observed that the flower extracts of *Adenium obesum* exhibited the highest radical scavenging activity. Fig.(3)NO is generated in biological tissues by specific nitric oxide synthases, which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction .[24]The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO . Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent.[25]It was observed that both the flower extracts of*Adenum obesium* inhibited nitric oxide in a dose dependent manner . Fig.(4)

CONCLUSION

Medicinal plants are used by humans since the beginning of human life on earth. From the present study, it can be concluded that , *Adenum obesium* flowers contain anthocyanins which are found to be responsible for its antioxidant activity. Well proved potent antioxidant activity of *Adenum obesium* flowers in the present study , strongly emphasize that it can be used as an accessable source of natural antioxidants with potential to provide protection against the management of diabetes associated oxidative stress induced complications.

ACKNOWLEDGEMENT

I am thankful to my guide Dr.N. Raghunandan for extending his support during the entire work.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs. 1995 Mar 1;49(3):345-61.
2. Sato M, Ramarathnam N, Suzuki Y, Ohkubo T, Takeuchi M, Ochi H. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. Journal of Agricultural and Food Chemistry. 1996 Jan 18;44(1):37-41.
3. Hussein MA. Antidiabetic and antioxidant activity of Jasonia montana extract in streptozotocin-induced diabetic rats. Saudi Pharmaceutical Journal. 2008 Jul;16(214-221).
4. Ames BN, Shigengo MK, Hagen TM. Oxidants and degenarative disease of ageing. Proceedings of Natural Academy of Sciences of the United States of America.1993; 90; 7915-7922.
5. Oberley L. Free radical and Diabetes. Free Rad Biol MED.1998; 5:113-19.
6. Hussein MA. Antidiabetic and antioxidant activity of Jasonia montana extract in streptozotocin-induced diabetic rats. Saudi Pharmaceutical Journal. 2008 Jul;16(214-221).
7. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological research. 2001 Jan 1;50(6):537-46.
8. Bayes JW, Thrope SR. The role of oxidative stress in diabetic complications.curr.opinn. 1997; 3: 277-284.
9. Cordell GA, Colvard MD. Natural products and traditional medicine: turning on a paradigm. Journal of natural products. 2012 Jan 10;75(3):514-25.
10. De Lima AA, Sussuchi EM, De Giovani WF. Electrochemical and antioxidant properties of anthocyanins and anthocyanidins. Croatica chemica acta. 2007 Mar 11;80(1):29-34.
11. Meister A. Glutathione-ascorbic acid antioxidant system in animals. Joul of Biological Chemistry-Paper Edition. 1994 Apr 1;269(13): 9397-400.
12. Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K. Regulation and function of ascorbate peroxidase isoenzymes. Journal of experimental Botany. 2002 May 15;53(372):1305-19.
13. Smith CM and Reynard AM. Essentials of pharmacology. 4ed. Philadelphia:WB Saunders;1995.

14. Saslis-Lagoudakis CH, Hawkins JA, Greenhill SJ, Pendry CA, Watson MF, Tuladhar-Douglas W, Baral SR, Savolainen V. The evolution of traditional knowledge: environment shapes medicinal plant use in Nepal. Proceedings of the Royal Society of London B: Biological Sciences. 2014 Apr 7;281(1780):20132768.
15. Dimmit M, Hanson C. The genus Adenium in cultivation. Part 1: A. obesum and A. multiflorum. Cactus Succulent J.(US). 1991;63:223-6.
16. Schmelzer GM and Gecrib-Fakimm A. Plant resources of tropical africa: Medicinal plants. 11ed. Netherlands: Backhuys; 2008.
17. Aebi H. Catalase. In Bergmeyer Hu (ed) Methods in enzymatic analysis. Academic press: New York. 1983;276-286.
18. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics. 1978 Feb 1;186(1):189-95.
19. Rao MN. Nitric oxide scavenging by curcuminoids. Journal of pharmacy and Pharmacology. 1997 Jan 1;49(1):105-7.
20. Garrat D C.Ameloiration of chronic ileitis by nitric oxide synthetase inhibition. The J Pharmac and Experi. 1999; 3: 456-458
21. Rigoulet M, Yoboue ED, Devin A. Mitochondrial ROS generation and its regulation: mechanisms involved in H2O2 signaling. Antioxidants & redox signaling. 2011 Feb 1;14(3):459-68.
22. Rupeshkumar M, Kavitha K, Haldar PK. Hypoglycemic effect of Andrographis echioides on streptozotocin induced experimental rats. J App Pharm. 2015;7:60-8.
23. Baumann J, Wurn G, Bruchlausen FV. prostaglandin synthetase inhibiting o 2-radical scavenging properties of some flavonoids and related phenolic compounds. Naunyn Schmiedebergs.1979; 307: R1-R77.
24. Bredt DS. Endogenous nitric oxide synthesis: biological functions and pathophysiology. Free radical research. 1999 Jan 1;31(6):577-96.
25. Marcocci L, Maguire JJ, Droylefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochemical and biophysical research communications. 1994 Jun 15;201(2):748-55.

Reviewers of this article

Dr.P.Sailaja Rao

Assistant professor
Department of pharmacology
86,Sri venkareshwara college
Madhapur
Hyderabad-500081

Prof.Dr. M.Ranga Priya, M.Pharm., Ph.D., R.Ph.

Professor , Dept of Pharmaceutics,Sun Institute Of Pharmaceutical Education & Research, Kakupall, Nellore Rural, Nellore, Andhra Pradesh 524346

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript