

## Antioxidant Activity of *Acacia tortilis* Polysaccharide in Streptozotocin-Nicotinamide Induced Diabetic Rats

Bhateja P K<sup>2</sup>, Singh R<sup>1\*</sup>

<sup>1</sup>Department of Pharmacology, M. M. University, Mullana, Ambala, Haryana.

<sup>2</sup>Department of Pharmacology, Bhojia Dental College and Hospitals, Bhud, Baddi, Himachal Pradesh, India-173205.

Available Online: 10<sup>th</sup> June, 2016

### ABSTRACT

The present study was undertaken to evaluate the antioxidant effect of aqueous extract of *Acacia tortilis* polysaccharide (AEATP) from gum exudates in (STZ)-NAD induced diabetic rats. Male albino wistar rats were divided into control, diabetic, diabetic rat+ glibenclamide, diabetic rat+250, 500, 1000 mg/kg of AEATP. Fasting blood glucose (FBG), serum insulin, tissue superoxide dismutase (SOD), catalase (CAT), GPx, GSH, and malondialdehyde (MDA) were measured in liver, kidney and pancreas. In vitro antioxidant levels were measured through DPPH, ABTS, and FRAP methods. Positive antioxidant activities of AEATP were confirmed by DPPH, ABTS and FRAP assays. Administration of STZ significantly increased fasting blood glucose level and oxidative stress by decreasing SOD, Catalase, GPx activities and GSH level whereas increasing MDA levels. AEATP showed significant reduction ( $p < 0.05$ ) in fasting blood glucose level compared to diabetic rats. Tissue SOD, Catalase, GPx activities and GSH level along with MDA levels were attenuated in diabetic rats by treatment with AEATP. These data suggested that AEATP possess antioxidant and hypoglycemic effects by reducing oxidative stress in diabetic rats.

**Keywords:** *Acacia tortilis*, Streptozotocin (STZ), Diabetes mellitus, Antioxidant.

### INTRODUCTION

Oxidative stress is considered the major etiological factor in diabetes by increasing lipid peroxidation. Oxidative stress is due to imbalance between reactive oxygen species (ROS) and antioxidant level and observed both in preclinical and clinical diabetes<sup>1</sup>. Oxidative stress in diabetes is due to activation of transcription factors and advanced glycosylated end products (AGEs) followed by overproduction of ROS that lead to tissue damage<sup>2</sup>. Superoxide radical, hydroxyl radical and hydrogen peroxide are the major reactive oxygen metabolites involved in complication of diabetes and are adjusted by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and non-enzymatic scavengers like reduced glutathione (GSH) both *in vivo* and *in vitro*. Several studies reported marked increase in ROS and decrease in antioxidant enzymes in diabetes<sup>3,4</sup>. Various studies have been documented to reduce the oxidative stress in patient with diabetes by supplementation with naturally occurring antioxidants<sup>5</sup> and parallel to reduce the cost, limitation and adverse effects; focus has been shifted towards the medicinal herbs for their safe and effective use. Recently a lot of medicinal herbs are being investigated for their role in pharmacotherapy of diabetes as antioxidant<sup>6</sup>. *Acacia tortilis* (Israeli babool and umbrella thorn) is widespread distributed around the globe (Africa, Algeria, Egypt, Asia, Israel, Somalia, Pakistan, and India). In India, this tree was introduced in 1958 from Israel<sup>7</sup>.

Various extracts of this plant have been documented in smooth muscle relaxing activity, effective in treatment of  $\alpha_2$ -adrenoceptor related diseases, antimicrobial activity, antiplasmodial, antileishmanial antiviral, antiasthmatic, hypotensive and diuretic property<sup>8-13</sup>. Various species of *Acacia* are reported to have antioxidant activity like *Acacia nilotica*, *Acacia podalyriifolia*, *Acacia catechu*, *Acacia leucophloea*, *Acacia melanoxylon*, *Acacia dealbata*, and *Acacia leucophloea*<sup>14-19</sup>. Previously numerous studies on polysaccharides from *Laminaria japonica*, *Agaricus bisporus*, *Astragalus mongholicus*, *Physalis alkekengi* and *Litchi Chinensi*<sup>20-24</sup> demonstrated the antioxidant activity. Free radicals produce oxidative stress which in turn causes development and progression of diabetes and its complications. Structural damage of various organs (liver, kidney, and pancreas) and mainly pancreatic  $\beta$ -cells takes place due to presence of reactive oxygen species (ROS) which is also result from non-enzymatic glycosylation and activation of the polyol pathway in diabetic condition<sup>25,26</sup>. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are the protective enzymes against ROS so antioxidants are useful in diabetes treatment. The major goals of antioxidant treatment have been to reduce oxidative stress by preventing or delaying the progression or reversing the complications of diabetes. We have already shown the anti-diabetic activity of aqueous extract of *Acacia tortilis* polysaccharide from gum exudates<sup>27</sup> but its antioxidant

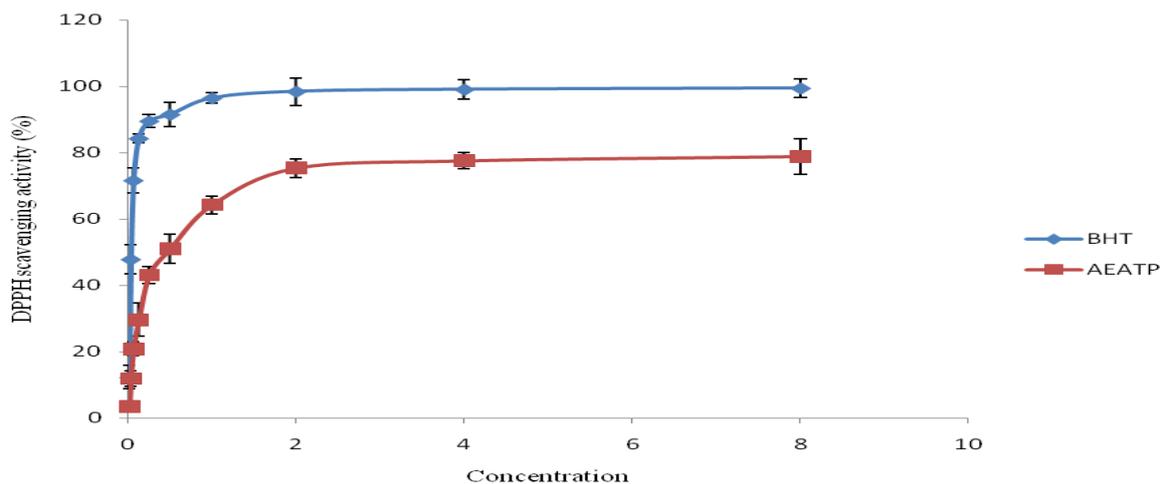


Figure 1: Effect of AEATP on DPPH radical scavenging activity. Data are shown as the mean  $\pm$  SD of 6 animals; The IC50 value of the AEATP was found 5.97 mg/ml.

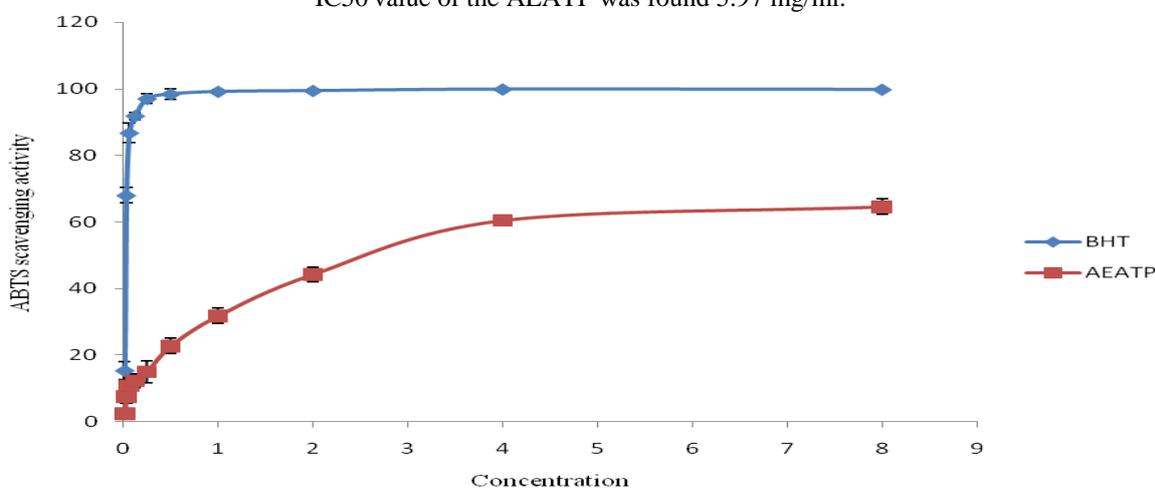


Figure 2: Effect of AEATP on ABTS scavenging. Data are shown as the mean  $\pm$  SD of 6 animals; The IC50 value of the AEATP was found 8.73 mg/ml

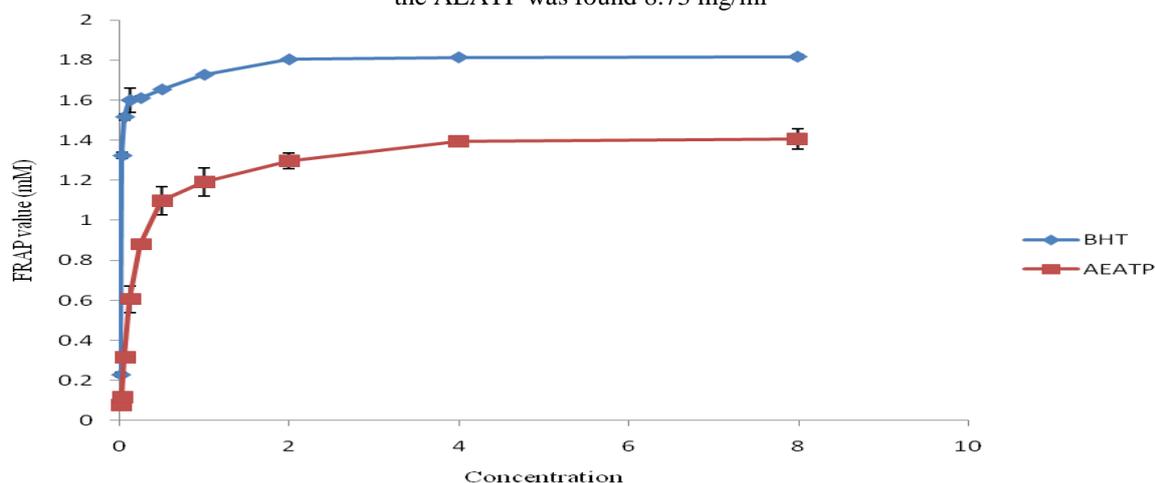


Figure 3: Effect of AEATP on FRAP Activity. Data are shown as the mean  $\pm$  SD of 6 animals.

property in Type-2 diabetes mellitus is still remain explored.

### MATERIAL AND METHODS

#### Chemicals

steptozotocin (STZ), Bovine serum albumin (BSA), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric

acid (TBA), reduced glutathione (GSH), were obtained from Sigma (St. Louis, MO, USA).

#### Plant Material

*Acacia tortilis* gum exudates from the stem and branches was collected from Central Arid Zone Research Institute Campus, Jodhpur, India.

#### Animals

Table 1: Antioxidant effect of AEATP on various enzymes in liver homogenate

Liver		SOD	Catalase	Lipid Peroxidation (MDA)	GPx	GSH
control	Basal	31.74± 1.90	168.44±11.52	6.18±1.75	0.952±0.06	41.06±3.05
	0 Day	31.58± 1.98	165.58±14.28	6.34±2.40	0.966±0.06	41.88±4.22
	6 <sup>th</sup> Week	31.34± 1.08	165.86±7.03	6.42±2.19	0.968±0.05	42.26±4.17
diabetic	Basal	32.74± 1.71	161.32±13.86	6.76±1.48	0.946±0.02	39.3±6.12
	0 Day	12.48± 1.08 a***	56.28±11.64 a***	19.08±5.79 a***	0.528±0.07 a***	11.4±4.87 a***
	6 <sup>th</sup> Week	11.64± 1.70 a***	46.26±12.21 a***	20.42±3.57 a***	0.43±0.03 a***	10.32±3.16 a***
Diabetic rats + Glimpiride	Basal	32.26± 2.20	164.26±10.93	6.78±2.31	0.966±0.10	40.14±6.10
	0 Day	13.68± 3.44 a***	58.28±10.11 a***	20.28±3.79 a***	0.562±0.07 a***	11.88±4.96 a***
	6 <sup>th</sup> Week	27.28± 1.79 b***	111.88±6.29 b***	12.62±4.19 b**	0.85±0.09 b***	31.38±1.84 b***
Diabetic rats + 250 mg/kg AEATP	Basal	31.28± 2.13	169.28±11.02	6.84±2.06	0.942±0.04	40.86±6.46
	0 Day	12.74± 3.33 a***	59.14±11.92 a***	19.34±5.58 a***	0.558±0.07 a***	11.24±4.93 a***
	6 <sup>th</sup> Week	15.94± 2.03 b*	43.92±7.65	16.28±3.69	0.564±0.06 b**	16.64±3.46
Diabetic rats + 500 mg/kg AEATP	Basal	32.16± 2.39	166.16±10.91	6.36±2.09	0.968±0.06	42.04±5.75
	0 Day	12.78± 3.58 a***	56.78±6.00 a***	19.98±4.49 a***	0.534±0.06 a***	10.6±3.72 a***
	6 <sup>th</sup> Week	18.98± 1.15 b***	63.36±8.06 b*	13.32±3.11 b**	0.576±0.05 b**	18.28±4.22 b**
Diabetic rats + 1000 mg/kg AEATP	Basal	31.06± 2.69	167.06±9.81	6.66±2.55	0.984±0.10	41.44±3.20
	0 Day	12.68± 3.72 a***	58.68±11.60 a***	19.48±5.20 a***	0.55±0.05 a***	11.26±3.45 a***
	6 <sup>th</sup> Week	22.88± 3.53 b***	94.44±7.78 b***	10.36±2.08 b***	0.68±0.05 b***	18.68±4.33 b**

Male albino Wistar rats (150–200 gm) were used in this study and experimental protocol was approved by Institutional Animal Ethics Committee. Animals were kept as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Chitkara College of Pharmacy Animal Facility Registration number: 1181/ab/08/CPCSEA). Animals were fed normal chow diet and *ad libitum* under controlled environmental condition of temperature (24–28° C), relative humidity 60– 70%, and natural light/dark cycle (12:12) and maintained on standard food pellets and tap water *ad libitum*.

*Experimental Protocol*

In experimental animals, diabetes was induced by intraperitoneal injection of Nicotinamide (230mg/kg) 15 min before streptozotocin (65mg/kg, i.p) administration<sup>28</sup>. The STZ was freshly prepared by dissolving in 0.1M citrate buffer, pH 4.5, and nicotinamide was prepared in normal saline. Diabetes mellitus was confirmed after 14 days of STZ administration when fasting blood glucose level had become constant above 250mg/dL. As STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ-treated rats were provided with 10% glucose solution after 6 hr for the next 24 hr to prevent fatal hypoglycemia<sup>29</sup>. Further, animals with blood glucose level above 250mg/dL were selected and divided into six groups comprising six animals in each group.

Group 1: Control

Group 2: Diabetic

Group 3: Diabetic + Glimpiride

Group 4: Diabetic rats + 250 mg/kg AEATP

Group 5: Diabetic rats + 500 mg/kg AEATP

Group 6: Diabetic rats + 1000 mg/kg AEATP

*Tissue homogenates*

After cervical dislocation, both kidneys were removed then left kidney was immediately preserved in 10% buffered formalin solution. Right kidney was washed with phosphate buffer, homogenized in 0.1 M phosphate buffer at pH 7.4 using a Teflon homogenizer then centrifuged at 9,000 g for 20 minutes to remove debris. The supernatant was further centrifuged at 15,000 g for 20 minutes at 40°C to get post mitochondrial supernatant. Liver of rats were removed and weighed then placed in 0.1 m phosphate buffer (pH 7.0), and homogenized on ice for 120 s with at 600 rpm further centrifuged at 1000 × g at 4°C for 10 min to remove tissue debris to get clear supernatant. Pancreas was dissected out immediately after sacrifice, washed in ice-cold saline and 10% homogenate in 0.1M Tris- HCL buffer (pH- 7.4) was prepared then centrifuged and supernatant was used for further analysis.

*In-vitro Antioxidant Activity*

*DPPH Assay*

The DPPH free radical scavenging activity of AEATP was determined by the method of Mohsen and Ammar<sup>30</sup> with slight modification. One mL of the tested samples at various concentrations (0.015–8 mg/mL) was added to ethanolic DPPH solution (3 mL, 0.1 mM). Discoloration was measured at 517 nm after incubation for 30 min at 30 °C in the dark. BHT was used as the positive control. The DPPH scavenging effect was calculated as follows:

DPPH scavenging effect (%) =  $[A_c - (A_s - A_b) / A_c] \times 100$   
 Where A, A<sub>b</sub> and A<sub>c</sub> were defined as absorbance of sample and DPPH, sample without DPPH, and DPPH without sample, respectively.

Table 2: Antioxidant effect of AEATP on various enzymes in kidney homogenate

Kidney		SOD	Catalase	Lipid Peroxidation (MDA)	GPx	GSH
control	Basal	22.46±2.28	25.22±3.78	5.82±1.70	0.71±0.04	36.94±5.39
	0 Day	20.52±2.88	23.46±3.42	5.94±1.68	0.71±0.09	35.76±4.72
	6 <sup>th</sup> Week	20.12± 2.76	24.38 ±4.23	5.64±1.55	0.76±0.03	35.38±3.96
diabetic	Basal	20.56±5.28	24.32±5.06	5.92±2.20	0.70±0.09	35.96±4.60
	0 Day	13.34±2.92 a**	11.08±5.02 a**	23.44±3.02 a***	0.42±0.03 a***	11.04±4.04 a***
	6 <sup>th</sup> Week	13.14±2.0 a***	10.86±2.27 a***	24.28±3.02 a***	0.39±0.07 a***	11.38±2.98 a***
Diabetic rats + Glimperiride	Basal	22.22±2.27	24.26±5.34	5.96±1.90	0.69±0.10	35.58±4.88
	0 Day	13.08±3.69 a**	10.88±4.89 a***	22.32±3.99 a***	0.40±0.078 a***	11.1±5.07 a***
	6 <sup>th</sup> Week	18.02±1.51 b**	18.84±4.87 b*	10.54±1.79 b***	0.68±0.09 b***	25.58±3.77 b***
Diabetic rats + 250 mg/kg AEATP	Basal	21.92±5.15	23.88±6.41	5.9±2.64	0.71±0.10	35.06±7.05
	0 Day	13.38±3.32 a**	10.34±5.24 a***	23.02±5.10 a***	0.42±0.10 a***	10.96±4.78 a***
	6 <sup>th</sup> Week	11.62±1.69	12.94±4.04	23.38±4.40	0.42±0.05	16.42±4.47
Diabetic rats + 500 mg/kg AEATP	Basal	21.8±2.35	23.76±5.42	5.22±1.77	0.71±0.08	36.1±9.26
	0 Day	13.14±2.95 a**	10.98±4.54 a**	22.94±3.53 a***	0.42±0.06 a***	11.06±4.27 a***
	6 <sup>th</sup> Week	16.86±2.72	17.82±2.73 b*	16.76±4.99 b*	0.51±0.03 b*	19.26±3.77 b*
Diabetic rats + 1000 mg/kg AEATP	Basal	20.76±1.09	24.66±4.77	5.82±1.04	0.69±0.08	36.64±6.17
	0 Day	13.2±2.99 a**	10.88±5.01 a***	22.82±5.54 a***	0.41±0.05 a***	11.88±3.87 a***
	6 <sup>th</sup> Week	17.46±2.55 b*	18.6±3.61 b*	16.24±3.90 b**	0.52±0.06 b*	20.64±4.09 b**

**ABTS Assay**

ABTS assay was carried out according to the method of Cai et al.<sup>31</sup>. The ABTS cation radical

Solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate and incubating in the dark at room temperature for 12 h. The ABTS cation radical solution was then diluted with water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. ABTS cation radical solution (3 mL) was added to the test samples (0.1 mL) of various concentrations (0.015–8 mg/mL) and mixed vigorously. The absorbance was measured at 734 nm after standing for 6 min. BHT was used as the positive control.

The ABTS scavenging effect was calculated as follows:

ABTS scavenging effect (%) =  $[A_c - (A_s - A_b) / A_c] \times 100$   
Where A<sub>s</sub>, A<sub>b</sub> and A<sub>c</sub> were defined as absorbance of sample and ABTS, sample without

ABTS, and ABTS without sample, respectively.

**Ferric Reducing Antioxidant Power (FRAP) Assay**

FRAP assay was carried out according to the method of Benzie and Strain<sup>32</sup> to measure the ability to reduce ferric ions. The fresh FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6, 10ml) with 100 ml of 10 mM TPTZ (tripyrindyl triazine) solution in HCl and ferric chloride solution (100 mL, 20 mM) in a ratio of 10:1:1 in volume. Blank reading was taken from freshly prepared FRAP reagent (37 °C) at 593 nm. Samples at different concentrations (0.6 ml, 0.015–8 mg/mL) were then added to FRAP reagent (4.5 ml) and incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. A standard curve was prepared using different concentrations of FeSO<sub>4</sub>·7H<sub>2</sub>O. The reducing ability was calculated from the linear calibration curve and expressed as mmol FeSO<sub>4</sub>·7H<sub>2</sub>O equivalents per gram of sample. BHT was used as the positive control.

**In-vivo Antioxidant Activity**

From all the tissue supernatants superoxide dismutase (SOD)<sup>33</sup>, catalase (CAT) activities<sup>34</sup>, GPx<sup>35</sup> glutathione (GSH)<sup>36</sup> and lipid peroxidation<sup>37</sup> in terms of MDA were estimated by standard methods. The protein content in liver, kidney and pancreatic tissues was measured by the method of Lowry et al.<sup>38</sup> with bovine serum albumin as standard.

**Estimation of Superoxide Dismutase (SOD)**

1.15 ml of distilled water, 50µl of homogenate, 1.2 ml of sodium pyrophosphate buffer of pH 8.3 (0.052M), 100µl post mitochondrial supernatant (186µM), 300µl of nitroblue tetrazolium (300µM) and 200µl of NADH (780µM) were added. 50µl of potassium phosphate buffer of pH 7.5 (0.1 M) was added for control reading replacing enzyme source. Optical density was measured in both control and test samples to calculate the enzyme activity. Where one unit of enzyme is defined as the amount of SOD required to give 50% maximal inhibition for the initial rate of NBT reduction<sup>33</sup>. Activity was expressed as unit enzyme/mg protein.

**Estimation of Catalase**

Catalase estimation was determined by disappearance of peroxide and analysed by spectrophotometer at 240 nm, the method was described by Sinha<sup>34</sup>. 1.9 ml of distilled water and 1.0ml of H<sub>2</sub>O<sub>2</sub> (0.059M) were added and incubated for 5 minutes at 25°C in spectrophotometer and absorbance was calculated at 240 nm. Homogenate (0.1 ml) was added to record the absorbance at 240 nm. One unit of CAT should turnover one micromole of hydrogen peroxide /min. The initial linear portion of the curve was used for change in the absorbance at 240 nm per min. Activity of CAT was measured in terms of U/mg protein.

**Estimation of Glutathione peroxidase (GPx)**

Estimation of Glutathione peroxidase (GPx) was carried out by Rotruck et al, using 0.2 ml 0.4 M phosphate buffer (pH 7.0), 0.1ml 10 mM sodium azide, 0.2 ml tissue

Table 3: Antioxidant effect of AEATP on various enzymes in pancreas homogenate

Pancreas		SOD	Catalase	Lipid Peroxidation (MDA)	GPx	GSH
control	Basal	16.2± 2.19	12.36±3.64	3.98±1.54	0.30±0.06	27.18±6.14
	0 Day	16.52± 3.03	11.78±4.10	3.64±1.20	0.31±0.06	27.64±7.48
	6 <sup>th</sup> Week	16.52± 3.03	12.46±1.68	3.42±1.43	0.31±0.07	27.46±4.53
diabetic	Basal	15.56± 2.85	11.8±4.90	3.9±1.97	0.30±0.06	27.42±6.79
	0 Day	8.64± 1.30 <sup>a***</sup>	6.28± 1.98 <sup>a**</sup>	17.2±4.63 <sup>a***</sup>	0.17±0.06 <sup>a**</sup>	13.98±4.26 <sup>a**</sup>
	6 <sup>th</sup> Week	8.28± 1.88 <sup>a***</sup>	6.52±1.24 <sup>a***</sup>	17.38±3.55 <sup>a***</sup>	0.15±0.02 <sup>a***</sup>	13.68±3.77 <sup>a***</sup>
Diabetic rats + Glimpiride	Basal	16.06±3.11	11.84±5.18	3.66±2.16	0.29±0.06	28.5±5.38
	0 Day	8.82± 1.74 <sup>a***</sup>	6.84±2.23 <sup>a*</sup>	16.36±5.39 <sup>a***</sup>	0.17±0.02 <sup>a***</sup>	13.3±5.48 <sup>a***</sup>
	6 <sup>th</sup> Week	14.78± 2.38 <sup>b***</sup>	10.32±2.14 <sup>b*</sup>	11.52±2.30 <sup>b**</sup>	0.27±0.04 <sup>b**</sup>	20.58±1.53 <sup>b**</sup>
Diabetic rats + 250 mg/kg AEATP	Basal	15.9± 3.02	12.12±4.31	3.96±1.67	0.31±0.05	27.4±7.09
	0 Day	8.82± 1.38 <sup>a***</sup>	6.38±1.91 <sup>a**</sup>	16.44±5.68 <sup>a***</sup>	0.17±0.05 <sup>a**</sup>	13.48±5.46 <sup>a**</sup>
	6 <sup>th</sup> Week	10.18± 2.73	9.08±2.19	12.52±3.40 <sup>b*</sup>	0.15±0.02	15.98±2.10
Diabetic rats + 500 mg/kg AEATP	Basal	16.52± 2.25	12.42±4.39	3.78±2.06	0.302±0.07	26.52±6.40
	0 Day	8.2± 1.59 <sup>a***</sup>	6.96±2.28 <sup>a*</sup>	18.32±5.14 <sup>a***</sup>	0.17±0.04 <sup>a***</sup>	13.4±3.84 <sup>a**</sup>
	6 <sup>th</sup> Week	13.52± 1.51 <sup>b**</sup>	10.58±2.25 <sup>b*</sup>	11.32±1.62 <sup>b**</sup>	0.26±0.05 <sup>b**</sup>	19.48±1.56 <sup>b*</sup>
Diabetic rats + 1000 mg/kg AEATP	Basal	15.46± 2.89	12.44±4.29	3.88±1.77	0.30±0.03	27.92±5.96
	0 Day	8.14± 1.56 <sup>a***</sup>	6.42±1.84 <sup>a*</sup>	17.84±3.51 <sup>a***</sup>	0.17±0.06 <sup>a***</sup>	13.86±5.22 <sup>a**</sup>
	6 <sup>th</sup> Week	14.08± 1.48 <sup>b**</sup>	10.66±1.71 <sup>b**</sup>	11.22±2.97 <sup>b**</sup>	0.26±0.04 <sup>b**</sup>	19.56±1.70 <sup>b*</sup>

homogenized in 0.4M phosphate buffer (pH 7.0), 0.2 ml reduced glutathione, 0.1 ml 0.2 mM hydrogen peroxide. This reaction mixture was incubated at 37 °C (10 min), 0.4 ml 10% TCA was added to stop the reaction and centrifuged at 3200 × g for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg DTNB in 100 ml 0.1% sodium nitrate). The activities were expressed as µg of GSH consumed/ min /mg protein<sup>35</sup>.

*Estimation of reduced glutathione (GSH)*

According to Moron et al,<sup>36</sup> the level of acid-soluble sulphhydryl group was determined which reflected GSH level, Reduced glutathione was used as a standard to calculate nmol of -SH content and can be determined spectrophotometrically at 412 nm against a reagent blank; the results were expressed as mM/mg protein.

*Estimation of MDA*

The malonyldialdehyde (MDA) was determined spectrophotometrically according to the method by Ohkawa et al<sup>37</sup>, 1979. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyle sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of homogenate. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration was expressed as nM of malondialdehyde /mg of protein.

*Sample collection and Estimation of Biochemical Analysis*

Rats were fasted overnight and blood samples were collected from retro orbital plexus under anesthesia for

biochemical estimation at basal, 0 day and 6 week of treatment of AEATP. Fasting blood glucose level of overnight fasted rats was measured using glucometer on the various time intervals of pharmacological interventions. HbA1c was estimated commercially available kits of Reckon Diagnostics Pvt. Ltd.

*Serum insulin estimation*

Serum insulin was measured by using ELISA kit (EMDMillipore-EZRMI-13 K) based on Sandwich ELISA technique and enzyme activity was measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products.

*Pancreatic insulin content*

The assay is. Pancreatic insulin content was extracted by taking 0.2 g pancreas portion with 5.0mL of ice-cold acid-alcohol in a centrifuge tube, homogenized, followed by sonication, stored at -20°C overnight, and finally centrifuged at 3000 rpm at 4°C for 15 minutes. The supernatant was transferred into a new centrifuge tube and stored at -20°C, while the pellet was subjected to extraction again. After extraction insulin level was measured at room temperature by ELISA assay kit<sup>39</sup>.

*Statistical methods*

Statistical analysis was performed using Graph pad Prism 6. Values are expressed as mean ± SEM. Blood glucose levels and biochemical parameters of different groups were statistically analyzed by two-way ANOVA followed by Tukey-Kramer multiple comparison test.

**RESULTS**

*In-vitro Antioxidant Activity of AEATP*

*DPPH Assay*

Free radical scavenging ability has been widely assessed by DPPH assay<sup>40,41</sup>. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is related to the

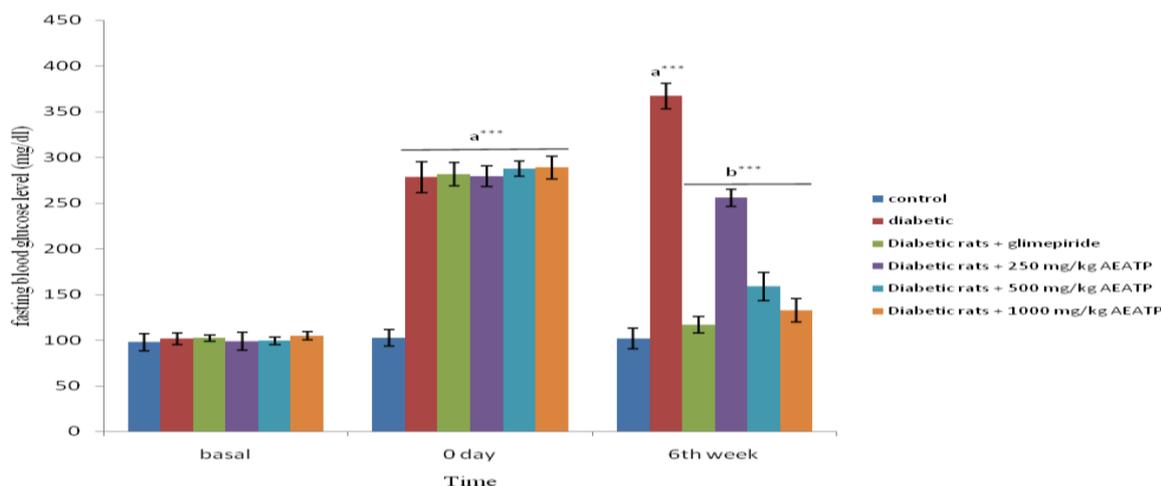


Figure 4: Effect of AEATP on fasting blood glucose level after 6-weeks treatment groups. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by Tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

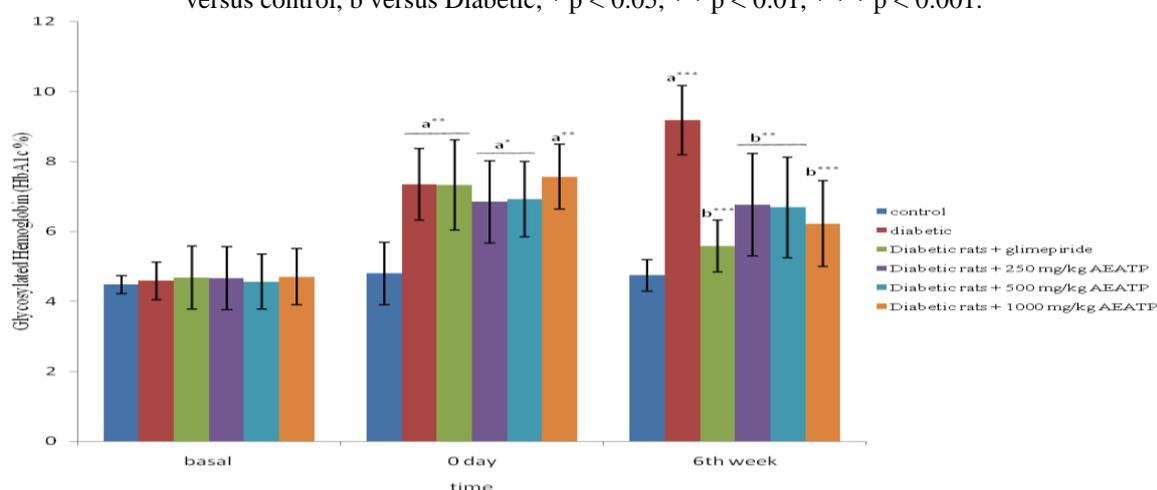


Figure 5: Effect of AEATP on Glycated haemoglobin (HbA1c) Level after 6-weeks treatment groups. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by Tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

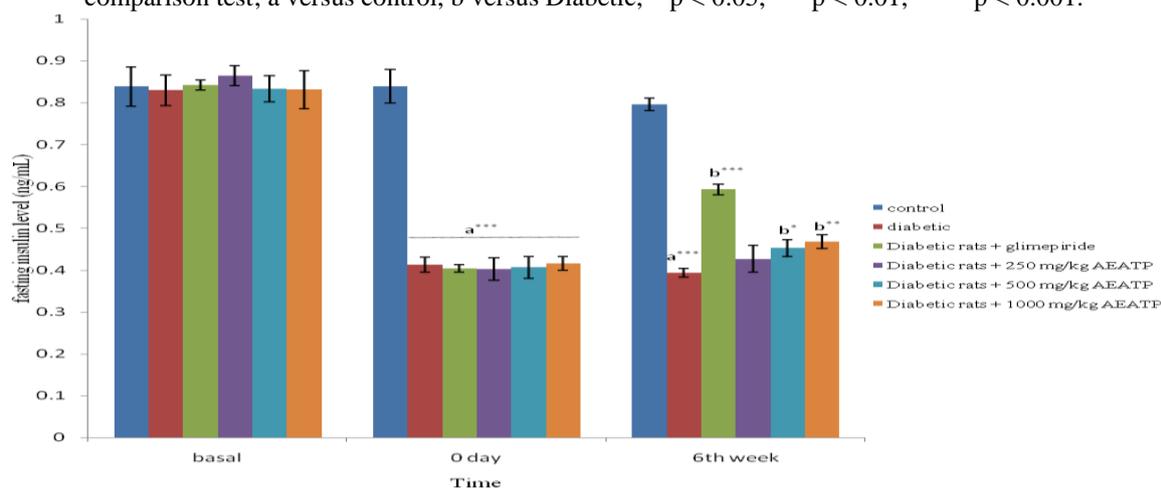


Figure 6: Effect of AEATP on fasting insulin level after 6-weeks treatment. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by Tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

number of electrons gained. Both AEATP and reference standard BHT exhibited DPPH radical scavenging ability. Dose range (0.015 to 8 mg/mL) % DPPH radical

scavenging effect (12.2% to 99.4%) of BHT was seen, similar effect produced by AEATP on % DPPH radical

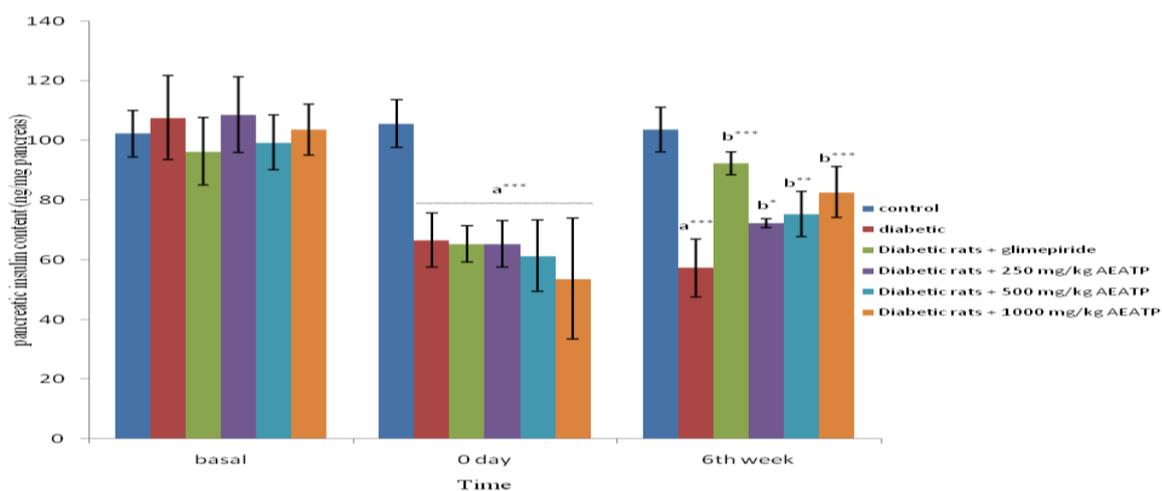


Figure 7: Effect of AEATP on pancreatic insulin content after 6-weeks treatment. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by Tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

scavenging i.e. 3.4% to 78.8% with  $IC_{50}$  value of 5.97 mg/mL (Figure 1).

#### ABTS Assay

ABTS assay is the another method to assess antioxidant activity<sup>42</sup>. The scavenging effect of AEATP increased according to concentration dependent manner. At concentrations from 0.015 to 8 mg/mL, the scavenging effect of BHT and AEATP ranged from 15.3% to 99.8%; 2.3% to 64.5% respectively, here  $IC_{50}$  value of AEATP was 8.73 mg/mL (Figure 2).

#### FRAP Assay

In this study, as the concentration of AEATP (0.015–8 mg/mL) is increased simultaneously increased colour intensity is also seen similar to BHT, due to formation of  $Fe^{2+}$ -TPTZ complex. AEATP exhibited an increase of FRAP values from 0.076 to 1.406 mM while, the FRAP values of BHT ranged from 0.23 to 1.81 mM (Figure 3).

#### In-vivo Antioxidant Activity of AEATP

**Effect on Superoxide Dismutase (SOD):** Diabetic rats shown significant decrease in SOD level in various tissue homogenate (liver, kidney and pancreas) at 0 day. Glimepiride and 1000 mg/kg of AEATP significantly increased SOD level in all tissue homogenate of diabetic group but 250 mg/kg of AEATP only significantly increased SOD level in liver but not in kidney and pancreas (Table 1,2 and 3). Antioxidant effect of AEATP on various enzymes in liver homogenate. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Effect on Catalase:** There was significant decrease in catalase activity in diabetic tissue homogenate (liver, kidney and pancreas) at 0 day. Glimepiride, 500 and 1000 mg/kg of AEATP except 250 mg/kg of AEATP significantly increased catalase level in all tissue homogenate of diabetic group after 6 weeks treatment (Table 1,2 and 3).

**Effect on Glutathione peroxidase (GPx):** STZ-induced diabetic rats shown significant decrease in GPx in various

tissue homogenate (liver, kidney and pancreas). Treatment with Glimepiride, 500 and 1000 mg/kg of AEATP significantly increased GPx level in all tissue homogenate of diabetic group. 250 mg/kg of AEATP only significantly increased GPx level in liver but not in kidney and pancreas (Table 1,2 and 3).

**Effect on reduced glutathione (GSH):** At 0 day of experimental protocol, GSH activity was significantly decreased in diabetic tissue homogenate (liver, kidney and pancreas). Glimepiride, 500 and 1000 mg/kg of AEATP except 250 mg/kg of AEATP significantly increased GSH activity in all tissue homogenate (Table 1, 2 and 3). Antioxidant effect of AEATP on various enzymes in kidney homogenate. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Effect on MDA:** MDA level was found to be significantly increased in various tissue homogenates (liver, kidney and pancreas) at 0 day. Treatment with Glimepiride, 500 and 1000 mg/kg of AEATP significantly decreased MDA level in all tissue homogenate of diabetic group (Table 1,2 and 3) after 6 weeks of treatment. Antioxidant effect of AEATP on various enzymes in pancreas homogenate. Data are shown as the mean  $\pm$  SD of 6 animals; Data was analyzed by using one way ANOVA followed by tukey-kramer multiple comparison test; a versus control, b versus Diabetic, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

#### Effect of AEATP on fasting blood glucose level:

The basal value of fasting blood glucose was found to be similar in all the groups. Fasting blood glucose level of control group ranged from  $97.8 \pm 9.41$  to  $102.6 \pm 8.90$  mg/dl in 6 weeks of study while there was a significant increase in fasting blood glucose level in STZ+NAD treated rat  $367.2 \pm 14.1$  mg/dl as compared to control group (Figure 4). After 6<sup>th</sup> week of treatment, significant reduction in blood glucose level was observed with glimepiride, 250, 500, 1000mg/kg of AEATP.

**Effect of AEATP on glycated hemoglobin (HbA1c):**

Glycated hemoglobin was found to be significantly elevated in STZ-induced diabetic group  $9.18 \pm 0.9\%$  as compared to control group  $4.74 \pm 0.4\%$  (Figure 5). After 6 weeks of treatment with glimepiride, 250, 500, 1000 mg/kg of AEATP glycated hemoglobin was found to be significantly attenuated as compared to diabetic group *i.e.*  $5.58 \pm 0.7\%$ ,  $6.76 \pm 1.4\%$ ,  $6.68 \pm 1.4\%$  and  $6.22 \pm 1.2\%$  respectively. Fasting plasma insulin was significantly decreased in STZ-induced diabetic rats ( $0.39 \pm 0.01$  ng/mL) as compared to control group ( $0.79 \pm 0.01$  ng/mL) (Figure 6). After administration of glimepiride, 500 and 1000 mg/kg of AEATP for 6 weeks, insulin level was significantly elevated *i.e.*  $0.59 \pm 0.01$ ,  $0.45 \pm 0.02$  and  $0.47 \pm 0.01$  ng/mL respectively as compared to diabetic group.

#### *Effect of AEATP on Pancreatic insulin content*

STZ-induced diabetic rats shown significant decrease in pancreatic insulin content ( $57.3 \pm 9.7$  ng/mg pancreas) as compared to control group ( $103.6 \pm 7.5$  ng/mg) (Figure 7). Glimepiride, 250, 500 and 1000 mg/kg of AEATP significantly increased pancreatic insulin content *i.e.*  $92.3 \pm 3.7$ ,  $72.3 \pm 1.5$ ,  $75.3 \pm 7.5$  and  $82.6 \pm 8.5$  as compared to diabetic control after 6 weeks treatment.

## DISCUSSION

The present study was designed to evaluate the antioxidant activity of AEATP in diabetic rats. Streptozotocin selectively damage the  $\beta$  cells of pancreas which in turn causes insulin deficiency which further causes increase serum glucose<sup>43</sup>. In previous report Streptozotocin along with nicotinamide increases blood glucose level<sup>29</sup> which in turn causes production of diabetes in rats and is attenuated with treatment of AEATP for 6 weeks by normalize the glucose level which is similar to standard drug glimepiride in our current report. Increased glycosylated hemoglobin [HbA1c] is another indicator of the diabetes which is formed due to non-enzymatically glycation of hemoglobin at N-terminal valine residue of the beta chain<sup>44</sup> and similar effect is seen in this study. Glycosylated hemoglobin decreased significantly when treated with aqueous extract of *Acacia tortilis* and maintains it at normal range. This piece of information strengthens the antidiabetic effect of AEATP. Streptozotocin mainly enters the  $\beta$  cells of pancreas through GLUT2 transporter and causes release of reactive oxygen species which result in damage of DNA fragment of  $\beta$  cells further decrease level of insulin.<sup>45, 46</sup> Our experiment also shows decrease level of plasma insulin and pancreatic insulin content which is well treated with AEATP and shows protective effects on  $\beta$  cells by acting against reactive oxygen species. Etiology of diabetes having involvement of various factors like increased oxygen free radical, alteration in antioxidant enzymes, nonenzymatic protein glycosylation, impaired glutathione metabolism and lipid peroxidation<sup>47</sup>. Various antioxidant enzyme level like SOD, catalase, GPx along with GSH are significantly decreased in tissue homogenate of liver, kidney and pancreas in diabetic rats. SOD converts the superoxide radicals into hydrogen peroxides and

molecular oxygen. Glycosylation of enzymes cause decrease level of SOD enzyme in diabetes<sup>48,49</sup>. Catalase involved in hydrogen peroxide metabolism to form oxygen and water. Hydrogen peroxide is a highly reactive and having detrimental effect on proteins, DNA and lipids<sup>50</sup>. catalase deficiency takes place in diabetes and cause damage of  $\beta$ -cell. Catalase produces the protective effects on pancreatic  $\beta$ -cells<sup>51</sup>. Glutathione peroxidase (GPx) is selenium based enzyme and produces GSH-dependent defense by reducing H<sub>2</sub>O<sub>2</sub> and lipoperoxides<sup>52</sup>. It is previously reported that the activity of antioxidant enzymes was reduced in liver and kidneys of diabetic rats<sup>53</sup>. Disturbed physiological level of oxygen and hydrogen peroxide in diabetes is maintained by antioxidant enzyme<sup>54</sup>. Treatment with aqueous extract of *Acacia tortilis* for 6 weeks increased the dismutation of oxygen radicals and eliminating peroxides by increasing level of SOD, catalase, GPx enzymes. When reduced glutathione is lowered in diabetes then it indicates increased oxidative stress<sup>55</sup>. Reduced glutathione is a tripeptide and act as an antioxidant by providing hydrogen molecule for reduction of hydrogen peroxide and lipoperoxides into water with the help of glutathione peroxidase<sup>56</sup>. In oxidative stress malondialdehyde (MDA) is considered as a primary biomarker of lipid damage<sup>57</sup>. Hydroperoxides have deleterious effects on cells and also react with metals [iron or copper] to form MDA, that produces peroxidative injury and become a major cause of diabetic complications in animal and human being<sup>58-60</sup>. In our study GSH is significantly increased in diabetic rats when treated with AEATP for 6 weeks. Reduction in the level of the MDA in various tissue homogenate after treatment shows the effective antioxidant activity of AEATP. DPPH is a stable radical and free radical scavenging activity of an antioxidant is best shown by this method. Free radical of DPPH is converted from colorful (deep purple color) to colorless at 517 nm by binding with electron or hydrogen radical of antioxidant to become a stable diamagnetic molecule<sup>61</sup>. The ABTS method is also used for evaluation of scavenging activity of antioxidant. Radical of ABTS reacts with potassium sulphate and give blue color. When this radical reacts with hydrogen atom donated by antioxidant then it converted into colorless radical form<sup>62</sup>. ABTS radical produces rapid and consistent degree of result due to its more reactivity than DPPH<sup>63</sup>. FRAP method is used to measure the reducing ability of an antioxidant by donating electron and convert ferric tripyridyltriazine [TPTZ-Fe (III)] complex to coloured (blue) ferrous tripyridyltriazine [TPTZ-Fe (II)] as in redox-linked colorimetric reaction<sup>32</sup>. Colour intensity corresponds to amount of the antioxidant in sample which is measured at 593 nm wavelength. All the *in vitro* study shows the antioxidant effect of AEATP. AEATP shows dose dependent increase in scavenging activity on free radicals in our present experimental protocol. Hyperglycemia triggers ROS production and lipid peroxidation<sup>64,65</sup>. Various Polysaccharide possesses potent antihyperglycemic effect by decreasing blood glucose level and corresponding decreased HbA1c<sup>66</sup>

further protection of pancreatic  $\beta$ -cells<sup>67</sup> contribute to its antidiabetic activity. In our study increased level of antioxidant enzymes in diabetic treated rats are due to aqueous extract of *Acacia tortilis*. This extract of *Acacia tortilis* is rich in polysaccharides which offered a good antioxidant potential and quash the deleterious effects of diabetes.

## CONCLUSION

In conclusion, our study shows that administration of AEATP reduces blood glucose which could be due to improvement in insulin secretion from pancreatic  $\beta$  cells. AEATP possesses antioxidant potential both in-vitro and in-vivo which may be used therapeutically for prevention of oxidative damage during diabetes. Therefore, it is concluded that the aqueous extract of *Acacia tortilis* polysaccharide possesses antidiabetic and antioxidant property for the management of diabetes.

## Abbreviations

DM, Diabetes mellitus; HbA1c, Glycated hemoglobin; NAD, Nicotinamide adenine dinucleotide; SD, Standard deviation; STZ, Streptozotocin; ANOVA, Analysis of variance; AEATP, Aqueous extract of *Acacia tortilis* polysaccharide

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