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ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF *ACACIA NILOTICA* LEAF ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

In the present study the antidiabetic and antioxidant activity of *Acacia nilotica* leaf against alloxan induced diabetics rats was performed. The rats treated with alloxan showed a significant increased in glucose level and altered level of lipid profile, antioxidants, heamoglobin, glycosylated heamoglobin and insulin. The mechanism underlying alloxan induced hyperglycemia in diabetes mellitus leads to over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. Six weeks administration of ethanolic leaf extract of *Acacia nilotica* (300mg/kg b.wt.) and standard drug glibenclamide (600µg/kg) to the diabetic induced rats resulted in significant ($p<0.05$) reduction in blood glucose level, restored heamoglobin, antioxidants and lipid profile as compared to untreated diabetic control rats. Study suggests that the *Acacia nilotica* leaves extract have significant hypoglycemic, antioxidant and hypolipidemic effect. The effect of *Acacia nilotica* leaves extract showed better response than standard drug glibenclamide. This potential activity of *Acacia nilotica* leaf might be due to the presence of its phytochemicals or the collective action of many active ingredients.

KEYWORDS: **Diabetes Mellitus, Alloxan, Antioxidant, Lipid profile, *Acacia nilotica***

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide. Currently there are over 150 million diabetics worldwide and this number is likely to increase 300 million or more by the year 2025 due to increase in sedentary lifestyle, consumption of energy rich diet, and obesity[1]. Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action. During diabetes or insulin resistance, increased oxidative glucose metabolism itself increases mitochondrial production of $O_2^{\bullet -}$, which will then be converted to $HO^{\bullet}$, and H_2O_2. Beyond glucose, ROS formation is also increased by FFAs (free fatty acids), through direct effects on mitochondria. It has been proposed that over expression and activity of a mitochondrial inner membrane uncoupling proteins (UCPs) contribute to an increase in superoxide formation under diabetic conditions[2]. Diabetic experimental animal models have shown that oxidative stress causes persistent and chronic hyperglycemia, thereby depleting the activities of the antioxidant defense system and otherwise promoting free-radical generation[3].The commercially available anti-diabetic drugs have potent activity but they have various severe adverse effects. Therefore, agents of natural origin with very little side effects are required as substitute for the chemical therapeutics. A vast range of these natural products and medicinal plants, including crude extracts and isolated compounds from plants can be used to regulate carbohydrate metabolism and prevent chemical induced diabetic[4]. In the recent decades [5,6], these have been vastly used in the management of diabetic due to the presence of several components with different antidiabetic and anti-oxidant effects. The medicinal value of the chosen plant, *Acacia nilotica* (Linn) leaf, is belonging to the family of Mimosaceae. *Acacia nilotica* leaf was commonly used for Chemoprventive, anitmutagenic, anti bacterial, anticancer, astringent, anti microbial activity. Tender leaves are used to treat diarrhea, Aphrodisiac, dressing of ulcers, anti-inflammatory and Alzheimer's diseases[7,8]. However, antidiabetic activity has not been evaluated. Therefore, the present study was to investigate the antidiabetic and antioxidant activity of ethanolic extract of *Acacia nilotica* leaf in alloxan induced diabetic rats.

MATERIALS AND METHODS

Animals

Male *albino* rats of W*istar* strain approximately weighing 180-200g were used in this study. They were healthy animals obtained from the Animal house (Regn. No. 743/03/abc/CPCSEA, dated on March 2003), PRIST University, Thanjavur. The experimental protocols were approved (PRIST / IAEC / Ph.D. BC - 03/ 2012 - 2013) by the IAEC, PRIST University, Thanjavur. The animals were housed in spacious polypropylene cages bedded with husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fiber (4.02%), Ash (8.02%) and sand silica (1.02%).

Collection and Preparation of Plant Material

The fresh plants of *Acacia nilotica* leaf were collected from natural wild habitats from outskirts dry land of city Thanjavur, Tamilnadu, India during the months of June 2013. The collected plant was identified by Dr. Jagadesan, Department of herbal and environmental science, Tamil University, Tamilnadu, India and deposited in the herbarium. The plants were washed thoroughly in running tap water to remove soil particles and adhered debris, then finally washed with sterile distilled water. The leaf of *Acacia nilotica* leaf was separated and dried under shade then ground well into fine powder. The powdered materials were stored in air tight containers until the time of use.

Preparation of extract

The powder material of *Acacia nilotica* leaves was macerated with 100% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45^{o}C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume with distilled water just before oral administration.

Alloxan induced diabetic rat

Diabetes was induced by an intraperitoneal injection of 150 mg/kg of alloxan monohydrate [9]. After 2 weeks, blood was taken from the eyes (Retro orbital fluxes) and glucose content was determined according to the method of Trinder[10]. The blood glucose levels of 200-280 mg/dl rats were considered as diabetic and were used for the study.

Experimental design

Body weights of the animals were recorded and they were divided into 5 groups of 6 animals each as follows. Group 1 Normal control rats fed with control diet served as a control. Group 2 Diabetic animals. Group 3 Diabetic animals co-administrated with *Acacia nilotica* leaf extract by oral gavage daily at a dose of 300 mg/kg body weight (based on effective dosage fixation studies) for 6 weeks. Group 4 Diabetic animals treated with standard drug glibenclamide at a dose of 600μg/kg body weight for 6 weeks. Group 5 administrated with *Acacia nilotica* leaf extract alone (Drug positive) by oral gavage daily at a dose of 300 mg/kg body weight for 6 weeks. On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with and without EDTA as an anticoagulant. Plasma and serum were separated for the estimation of various biochemical parameters.

Biochemical estimation

Glucose was estimated by method of Trinder[10].Haemoglobin was estimated by Cyanmethaemoglobin method[11]. Glycosylated haemoglobin was estimated by using the method of Trivelli *et al.* [12]. Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust [13]. Reduced glutathione was estimated by the method of Moron *et al* [14]. The level of ascorbic acid and α-tocopherol were estimated by the method of Omaye *et al*[15] and Baker *et al*[16.] Superoxide dismutase, catalase and glutathione peroxidase activity were determined by the procedure of Kakkar *et al* [17], Beers and Sizer[18] and Rotruck *et al* [19] respectively. Cholesterol and HDL cholesterol were estimated by Allain *et al*[20]. Triglyceride was determined by the method of Werner *et al*[21]. LDL and VLDL cholesterol were calculated as per Friedewald's[22] equation. Plasma insulin was assayed by the solid phase system amplified sensitivity immunoassay using reagent kits obtained from Medgenix-INS-ELISA, Biosource, Europe S.A., Belgium[23].

Statistical Analysis

Values were expressed as Mean $\pm$ SD for six rats in the each group and statistical significant differences between Mean values were determined by one way analysis of variance (ANOVA) followed by the Dunn's multiple comparisons. The results were statistically analyzed and $p< 0.05$ was considered to be significant.

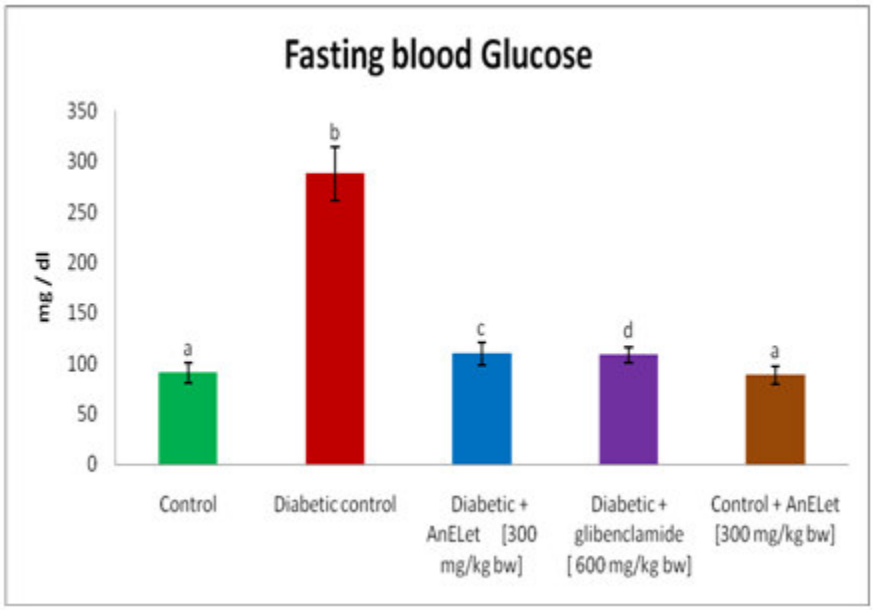
RESULTS

Figure 1 depicts the Mean body weight of control and experimental rats. The body weight was significantly decreased in alloxan treated diabetic rats as compared to control rats. Oral administration of ethanolic *Acacia nilotica* leaf extract at a dose of 300 mg/kg bw significantly increased the body weight in diabetic rats. The AnELet showed similar effect to that of glibenclamide. Statistically non-significant difference was observed between control rats and rats treated with AnELet alone. Figure 1 and 2 depicts the blood glucose and plasma insulin level in control and experimental rats.. Blood glucose level was significantly increased, whereas plasma insulin was significantly decreased in diabetic rats as compared to control rats. However, the levels of blood

glucose and plasma insulin were returned to near normal range in diabetic rats treated with ethanolic extract of *Acacia nilotica* leaves (AnELet) and diabetic rats treated with glibenclamide. *Wistar* rats treated with AnELet alone showed no significant difference in blood glucose and plasma insulin levels as compared to control rats.Figure 3 and 4 depicts the total haemoglobin and glycosylated haemoglobin in control and experimental rats in each group. Glycosylated hemoglobin level was significantly increased whereas total haemoglobin was decreased in diabetic rats as compared to control rats. However, the level of total haemoglobin and glycosylated haemoglobin were returned to near normal range in diabetic rats treated with ethanolic leaf extract of *Acacia nilotica* and diabetic rats treated with glibenclamide. *Wistar* rats treated with ethanolic extract of *Acacia nilotica*leaves alone showed no significant difference in total haemoglobin and glycosylated haemoglobin levels as compared to control rats.Figure 5 - 9 depicts the levels of cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C, in plasma of control and experimental rats in each group. LDL-C and VLDL-C levels were significantly increased, whereas HDL-C was decreased in diabetic rats as compared to control rats. However, the level of cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C was returned to near normal range in diabetic rats treated with ethanolic extract of *Acacia nilotica* leaves. The AnELet showed a similar effect to that of glibenclamide. Rats treated with *Acacia nilotica* leaves extract alone showed a non significant difference in cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C levels as compared to control rats.Figure 10 depicts the levels of TBARS in plasma of control and experimental rats in each group. TBARS level in plasma was significantly increased in alloxan induced diabetic rats as compared to control rats. Treatment of alloxan induced diabetic rats with ethanolic extract of *Acacia nilotica* leaves for 45 days / 6 weeks resulted in a marked decrease in plasma TBARS. The AnELet showed similar effect to that of glibenclamide. Statistically no significant difference was noticed to TBARS levels in rats treated with ethanolic extract of *Acacia nilotica* leaves alone as compared to control rats. Figures 11-13 show the activities of enzymatic antioxidants (SOD, CAT, GPx) in plasma of control and experimental rats in each group. SOD, CAT and GPx activities were significantly decreased in alloxan induced diabetic rats as compared to control rats. Treatment of alloxan induced diabetic rats with ethnaolic extract of *Acacia nilotica* leaves for 45 days resulted in a marked increase in SOD, CAT and GPx activities as compared to alloxan induced diabetic rats. The activities of SOD, CAT and GPx in diabetic rats treated with AnELet showed showed similar effect to that of glibenclamide.. Statistically no significant difference was noticed in enzymatic antioxidants activities in rats treated with ethanolic extract of *Acacia nilotica* leaves alone as compared to control rats.Figures 14-16 show the levels of non-enzymatic antioxidants (Vitamin C, Vitamin E and glutathione) in plasma of control and experimental rats in each group.. The levels of Vitamin C, Vitamin E and glutatione were returned to near normal range in diabetic rats treated with ethanolic extract of *Acacia nilotica* leaves and diabetic rats treated with glibenclamide. Rats treated with ethanolic extract of *Acacia nilotica* leaves alone showed a non significant difference in Vitamin-C, Vitamin-E and glutathione levels as compared to control rats.

Figure 1

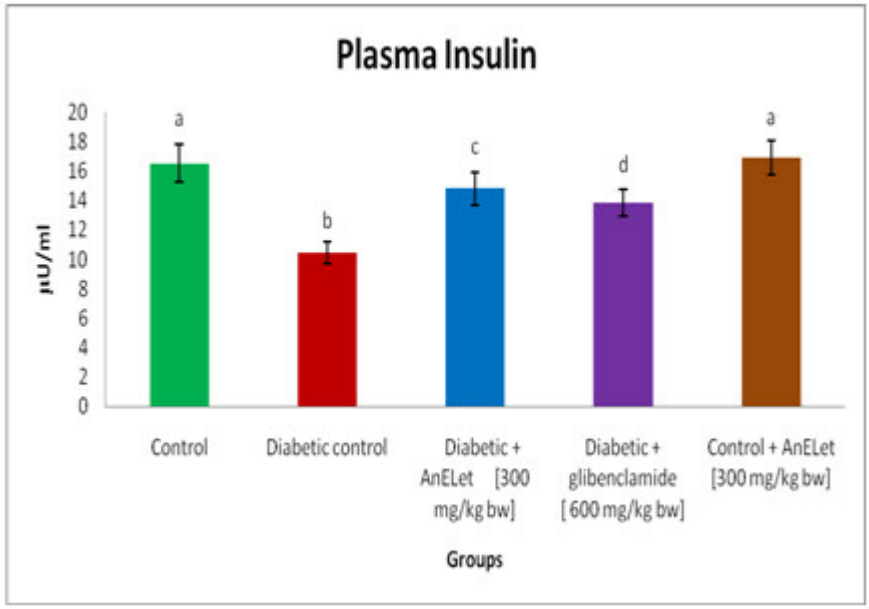
Effect of Acacia nilotica leaf on fasting blood glucose in experimental rats



Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT)AnELet – Acacia nilotica* ethanolic *leaf extract

Figure 2

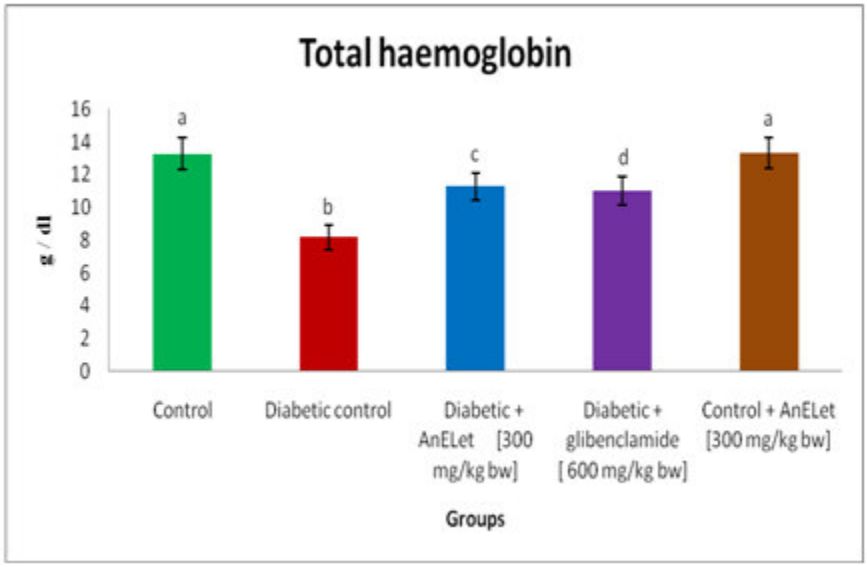
Effect of Acacia nilotica leaf on plasma insulin in experimental rats



Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica* ethanolic *leaf extract

Figure 3

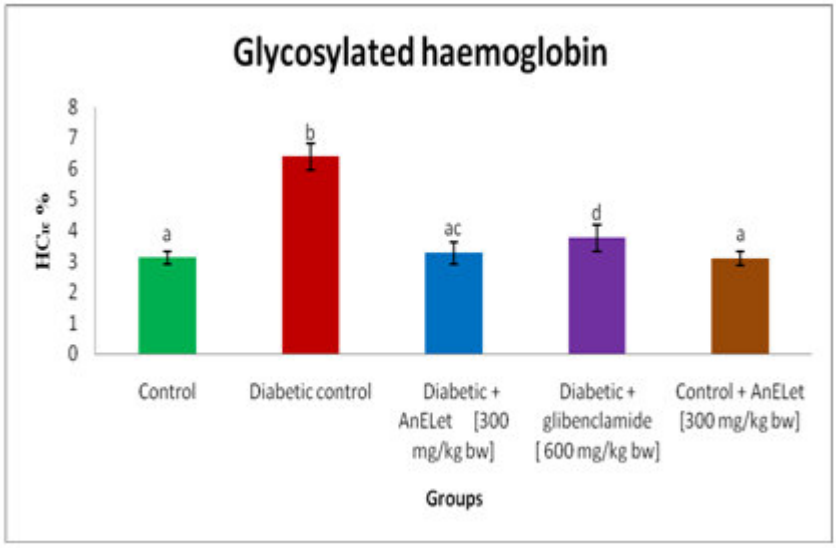
Effect of Acacia nilotica leaf on total haemoglobin in experimental rats



Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript Letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

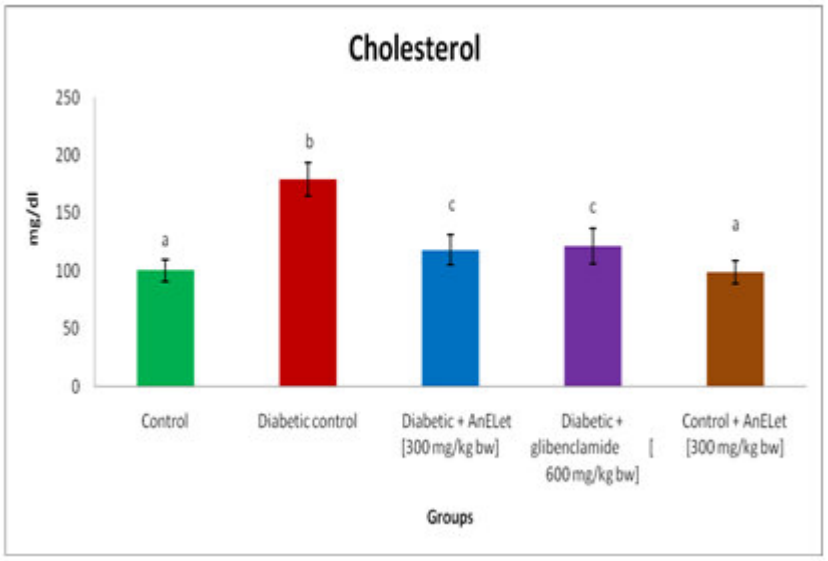
Figure 4

Effect of Acacia nilotica leaf on glycosylated haemoglobin in experimental rats



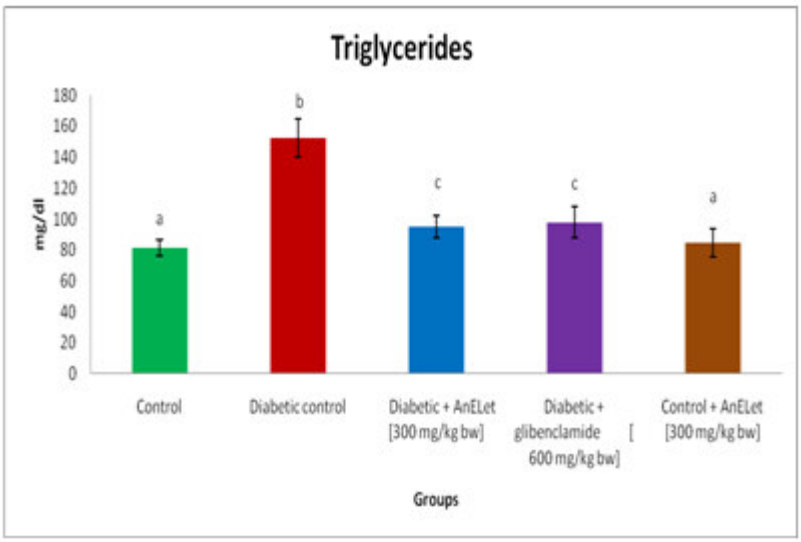
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 5
Effect of Acacia nilotica leaf on cholesterol in experimental rats



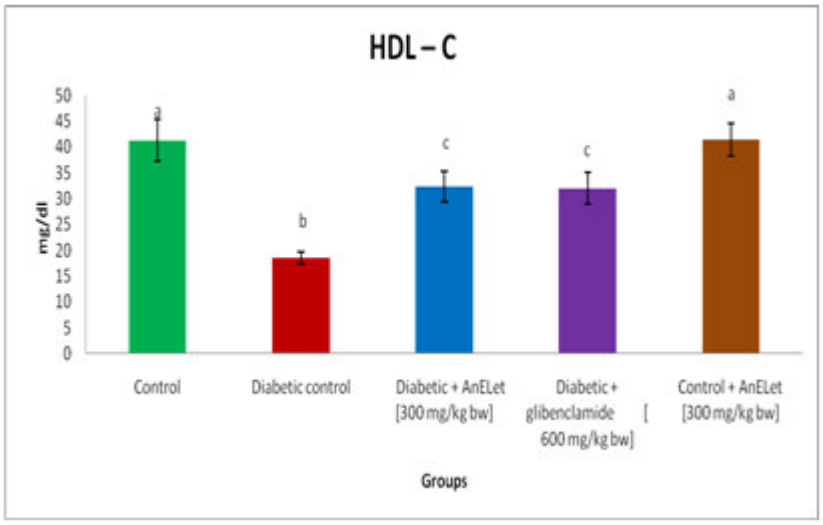
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 6
Effect of Acacia nilotica leaf on triglycerides in experimental rats



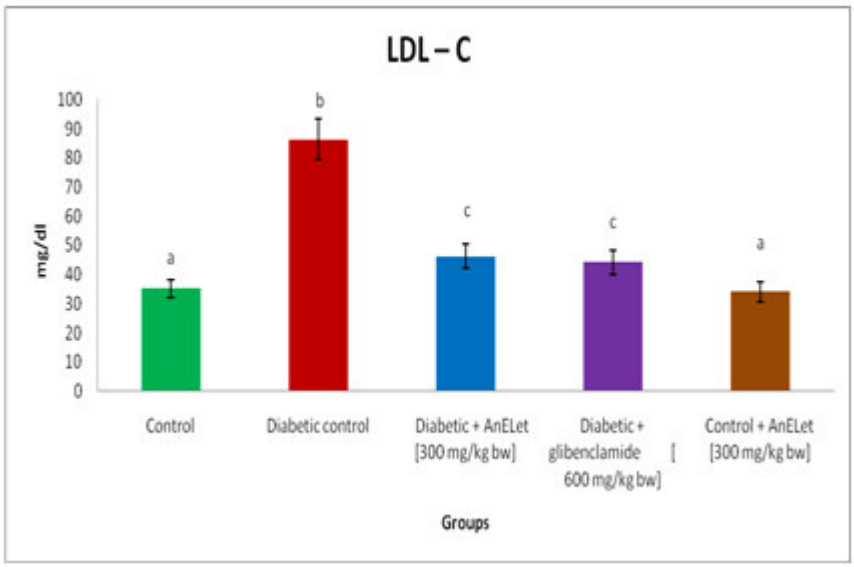
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 7
Effect of Acacia nilotica leaf on HDL-cholesterol in experimental rats



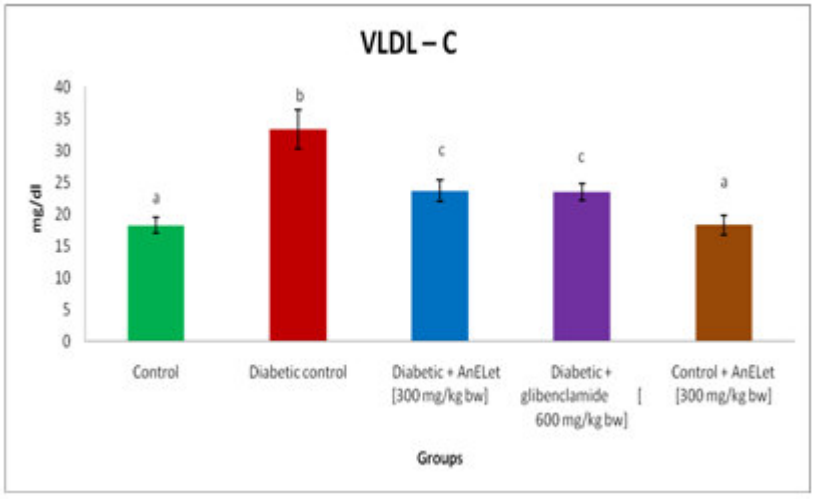
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ Significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 8
Effect of Acacia nilotica leaf on LDL-cholesterol in experimental rats



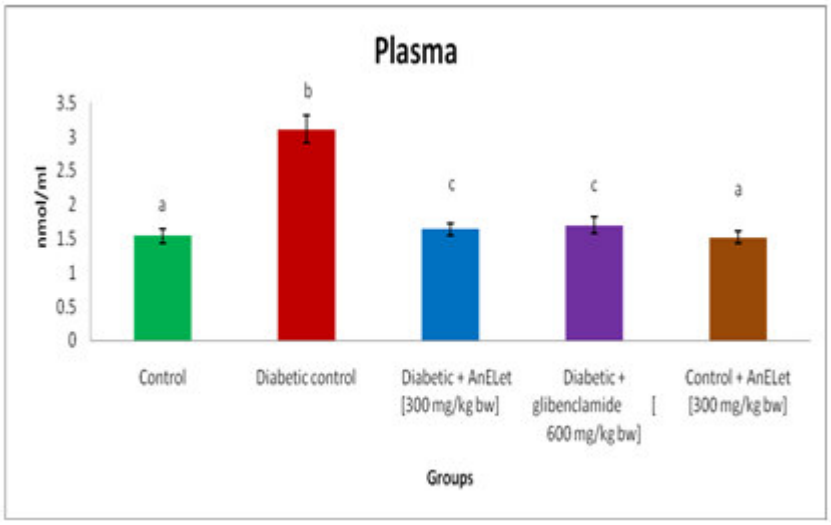
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ Significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 9
Effect of Acacia nilotica leaf on VLDL-cholesterol in experimental rats



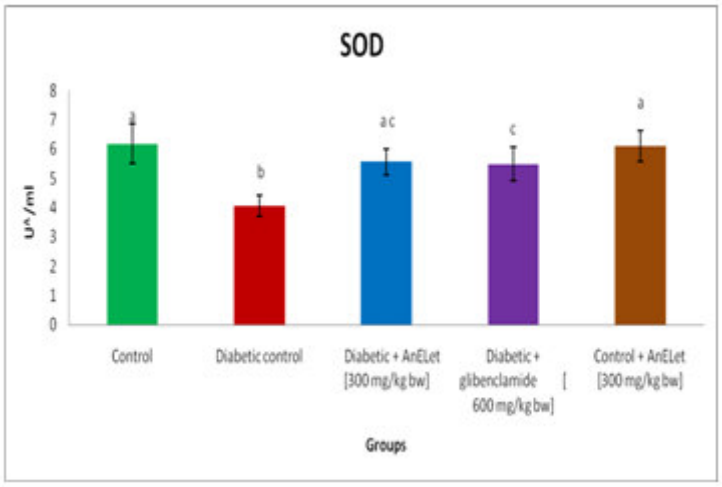
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 10
Effect of Acacia nilotica leaf on TBARS in experimental rats



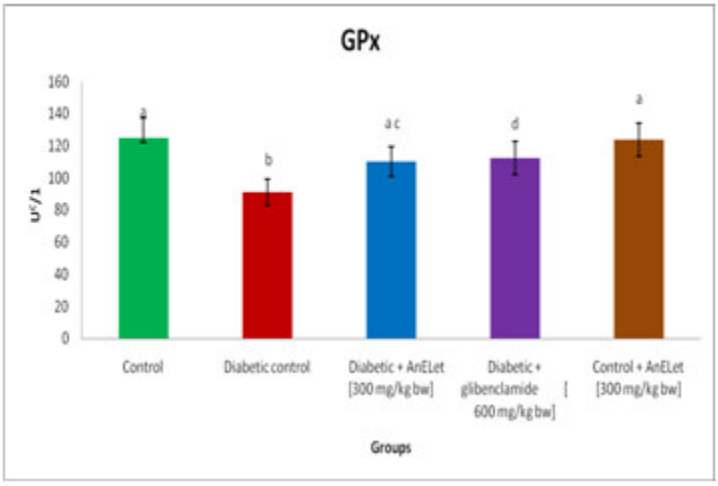
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 11
Effect of Acacia nilotica leaf on SOD activity in experimental rats



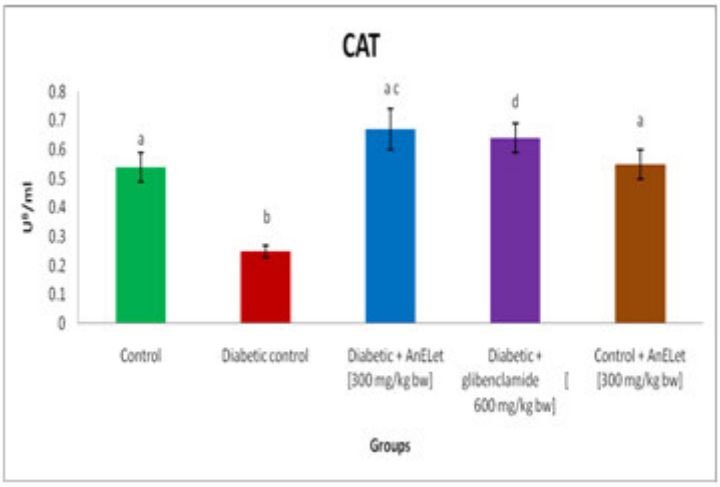
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 12
Effect of *Acacia nilotica* leaf on GPx activity in experimental rats



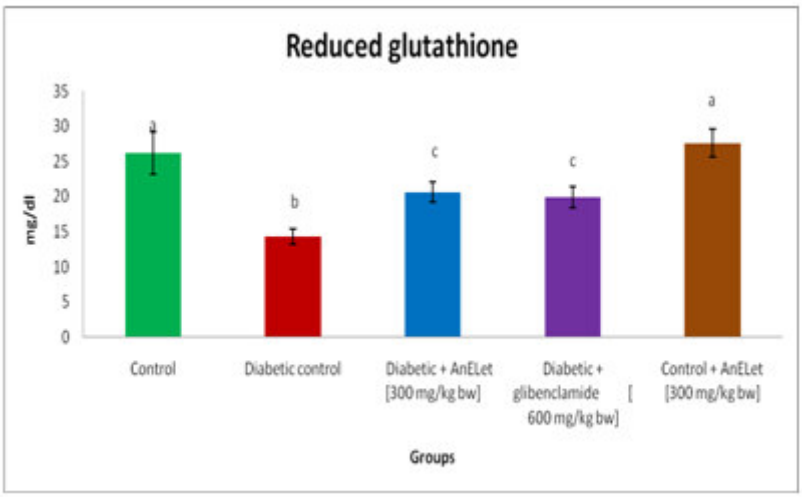
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 13
Effect of Acacia nilotica leaf on CAT activity in experimental rats



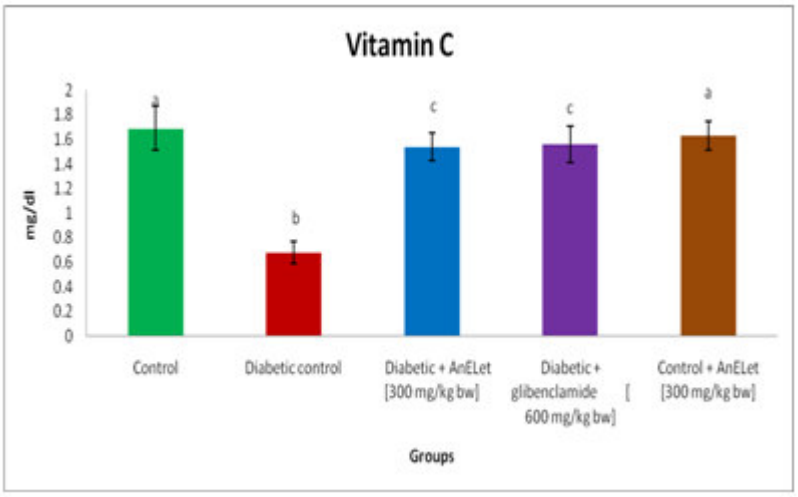
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 14
Effect of Acacia nilotica leaf on GSH (Reduced glutathione) in experimental rats



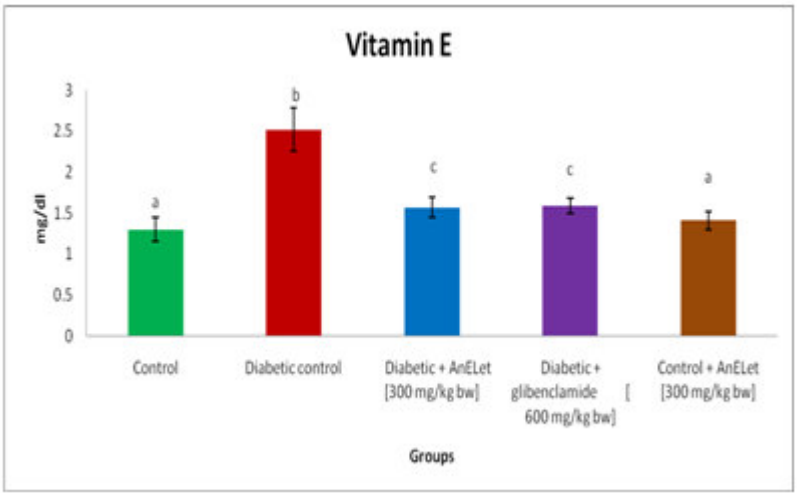
Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 15
Effect of Acacia nilotica leaf on Vitamin C in experimental rats



Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

Figure 16
Effect of Acacia nilotica leaf on Vitamin E in experimental rats



Values are given as Mean ± SD (n=6 rats) Values not sharing a common superscript letter differ significantly at P< 0.05 (DMRT) AnELet – Acacia nilotica ethanolic leaf extract

DISCUSSION

Alloxan diabetes is caused by the selective pancreatic beta cell toxicity of this compound [24,25]. In order to destroy insulin-producing cells and to induce a state of insulin dependent diabetes mellitus. The molecular shape of alloxan is similar to the glucose molecule, must be taken up into the cell via the low-affinity glucose transporter (GLUT2) in the plasma membrane [26] and is therefore toxic [27]. Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in alloxan-induced diabetes to compare the efficacy of a variety of antihyperglycemic compounds. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin will be secreted. Insulin is the dominant hormone which influencing the regulation of glucose metabolism. One of the major effects of

insulin is to enhance overall glucose disposal by stimulation of glucose uptake into the target tissues [28]. In the present study, there was a significant elevation in blood glucose and decreased insulin level in the diabetic control group as compared with normal animals. The *Acacia nilotica* leaves treated group exhibited significant reduction in fasting plasma glucose and increased insulin levels as compared to the diabetic control group. Reduction of insulin in alloxan induced diabetic results in enhanced the production of glucose by Means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus[29]. The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e., catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins [30,31]. In the present study, diabetic rats treated with *Acacia nilotica* showed an increase in bodyweight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis. The ability of *Acacia nilotica* leaf extract is effectively controlling the increase in blood glucose levels in the diabetic group of rats and increasing the body weight maybe attributed to its antihyperglycemic activity. Present finding is in agreement with Subramaniam *et al*.[32], Ayoola *et al*.,[33] and Sharma and Garg studies[34]. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens[35]. Glycosylated haemoglobin (HbA1) was found to increase in patients with diabetes mellitus to approximately 16% [36] and the amount of increase is directly proportional to the blood glucose level [37]. During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated hemoglobin... Therefore, the total haemoglobin levels decrease in alloxan diabetic rats [38]. Administration of *Acacia nilotica* leaf reversed the total haemoglobin and glyHb levels in alloxan diabetic rats.The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [39,40] and such an elevation represents a risk factor for coronary heart disease [9]. Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease [41]. A marked increase in total cholesterol and decrease in HDL cholesterol has been observed in diabetic control rats. Insulin deficiency results in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia [31]. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [9].There was a significant control of the levels of serum lipids in *Acacia nilotica* leaf treated diabetic rats. In diabetes, LDL and VLDL carry cholesterol to the peripheral tissues where it is deposited, where as HDL transports cholesterol from peripheral tissues to the liver and thus aids its excretion. Hence increase in LDL and VLDL is atherogenic [42]). The atherogenic and coronary index was also increased in this study. Administration of alcoholic extract of *Marsilea quadrifolia* stem and leaf normalized plasma lipids, secondary to the diabetic state. This study corroborate with Edwin *et al.,*[43] studies. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose [44]. The regression of the diabetic state on *Acacia nilotica* leaf administration increases the utilization of glucose, thereby depressing the mobilization of fat.Alloxan [1H,3H]-pyrimidinetetrone) can generate “reactive oxygen species” (ROS) in a cyclic reaction between this substance and its reduction product, dialuric acid (AH2) [45,46]. It has therefore been assumed that the beta cell toxic action of alloxan is initiated by free radicals formed intracellularly in this redox reaction. Autoxidation of dialuric acid has been shown to generate superoxide radicals ($O_2^{\bullet}-$) and hydrogen peroxide (H_2O_2) and, in the presence of a suitable catalyst, hydroxyl radicals ($^{\bullet}OH$). The autoxidation of dialuric acid involves the intermediate formation of the alloxan radical ($^{\bullet}AH$)[47,48]. Reduction of alloxan to dialuric acid in the cell requires the presence of a suitable thiol,

typically the tripeptide glutathione (GSH). Other intracellular thiols, present at lower concentrations in the cell, such as the monothiol cysteine and other thiols and dithiols, should also be suitable reducing agents and thus contribute, although to a lesser extent, to alloxan reduction[49].The major oxidation pathway of dialuric acid is an $O_2^{\bullet}$−dependent chain reaction, which is inhibited by superoxide dismutase (SOD). In the presence of SOD, an autocatalytic process involving the interaction between dialuric acid and alloxan becomes important[50] while in the presence of a transition metal, a third oxidation mechanism, dependent on H_2O_2, has been identified [51]. This latter step is inhibited by the hydrogen peroxide-inactivating enzyme catalase [46]. The other hydrogen peroxide-inactivating enzyme, glutathione peroxidase, should be able to act in a similar manner.The generation of free radicals may lead to lipid peroxidation in diabetes mellitus [52]. In the present study, the TBARS levels, a lipid peroxidation product and a marker of oxidative stress were elevated significantly in diabetic animals. Treatment with *Acacia nilotica* leaf significantly decreased the TBARS levels. Associated with the changes in lipid peroxidation, diabetic animals showed decreased activity of the key antioxidant enzymes viz .SOD, GPx, CAT and decreased content of non enzymatic antioxidants reduced GSH, Vitamin C and Vitamin E , which play an important role in scavenging the toxic intermediates of in complete oxidation. A decrease in the activity of these enzymes due to inactivation by free radical produced from alloxan detoxification. It can lead to an excess availability of superoxide anion ($O_2^{\bullet}$) and hydrogen peroxide in the biological systems, which in turn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation [53]. Glutathione is an important antioxidant that functions directly in elimination of toxic peroxides and aldehydes and indirectly in maintaining Vitamins C and E in its reduced and functional forms. Vitamin C deficiency results in decreased plasma GSH, Vitamin E supplementation increases plasma GSH. The observed decline in glutathione level may contribute to the decrease in Vitamin C as well Vitamin E concentration in diabetic rats The *Acacia nilotica* leaf treatment increased the antioxidants and may thereby help to control free radicals, as *Acacia nilotica* leaf has been reported to be rich in flavonoids and phenolic compounds, well-known antioxidants and also to possess *in vitro* free radical scavenging and antioxidant activity [7].The results of the above data conclude that *Acacia nilotica* leaf has blood glucose lowering action in diabetic induced animals and also found to have hypolipidemic and antioxidant activities, therefore it has an findings have given scientific evidence to the traditional use of *Acacia nilotica* leaf in the treatment of diabetes.

ACKNOWLEDGEMENT

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