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Protective effect of *Andrographis paniculata* on hyperglycemic mediated oxidative damage in renal tissues of diabetic rats

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ABSTRACT

The aim of this study is to evaluate the protective effect of ethanol extract of *Andrographis paniculata* (*A. paniculata*) on hyperglycemic mediated oxidative damage in the renal tissues of experimental diabetic rats and to determine the potential bioactive components of *A. paniculata* ethanol extract was scrutinized using GC-MS techniques. Diabetes was induced in rats by streptozotocin. Experimental period, the animal body weight, blood glucose, urea, serum proteins, cholesterol, antioxidant key enzymes and histological changes in kidneys were determined. The untreated diabetic and *A. paniculata* treated diabetic groups lost weight and consumed less quantity of food compared to the normal group. We noted decrease in blood glucose in the ethanol extract of *A. paniculata* treated diabetic group compared to normal and glibenclamide treated groups. The serum protein level was increase and cholesterol and urea levels were significantly ($P<0.01$) reduced in the *A. paniculata* extract treated group compared to the normal group. The levels of lipid peroxides in Plasma and kidney tissues were found to be elevated and kidney tissue enzymes of Superoxide dismutase, Catalase and Glutathione peroxidase were found to be decreased in streptozotocin induced diabetic rats when compared to normal. After treatment of *A. paniculata*, it brings back to significantly ($P<0.01$) near normal levels. Diabetes associated with marked histological changes in the kidney over a period of 32 days as revealed by tubular epithelial hypertrophy, glomerulosclerosis and glycogen accumulation. Treatment with *A. paniculata* extract afforded significant protection from renal damage whereas tubular damage was more pronounced in rats treated with glibenclamide. According to traditional indigenous medicinal systems of India this plant has got several medicinal effects without producing any severe side effects. This plant could be very well used as prevention of hyperglycemic complications and tissues damaging through the oxidation.

Keywords: *Andrographis paniculata*, Diabetes mellitus, Streptozotocin, GC-MS techniques.

INTRODUCTION

Diabetes mellitus is considered one of the main threats to human health in the 21st century. In developing countries, the prevalence of diabetes is increasing, where there are, as estimated by the World Health Organization (WHO), around 70 million people suffering from diabetes mellitus [1]. Changes in human activities and lifestyle over the last century have resulted in a remarkable increase in the incidence of diabetes worldwide [2]. Diabetes is a metabolic disorder or a chronic condition where the sugar levels in blood are high. [3]. If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death [4]. The reasons behind this projected increase in prevalence rate are due to urbanization, westernization and their associated lifestyle changes, increase in life expectancy at birth, physical inactivity and obesity and possibly a genetic predisposition [5,6], Age, ethnic, regional and racial differences have also been found to play a role for the diabetic incidence in heterogeneous populations within the same area [7,8].

Oxidative stress and oxidative damage to tissues are common end points of chronic diseases, such as diabetes, atherosclerosis and rheumatoid arthritis. Increased oxidative stress induced by hyperglycemia may contribute to the pathogenesis of diabetic complications. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Antioxidants give protection against oxidative attack by decreasing oxygen concentration, intercepting singlet oxygen, to avoiding first chain initiation by scavenging initial radicals, binding of metal ion catalysts, decaying the primary products of oxidation to non radical compounds and chain breaking to prevent constant hydrogen removal from substrates. Antioxidants react with radicals and other reactive species faster than biological substrates, thus protecting biological targets from oxidative damage. Furthermore the resulting anti oxidant radical possess high stability that is the antioxidant radical interrupts. Many plant-derived substances, collectively termed “phytonutrients,” or “phytochemicals,” are becoming increasingly known for their

antioxidant activity. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in the treatment of diabetes mellitus.

Andrographis paniculata (Burm. f) Nees., also called as Kalmegh or "King of Bitters" belongs to the family *Acanthaceae* is a herbaceous plant [9,10]. Mostly leaves and roots have been traditionally used over centuries for different remedy for a wide spectrum of ailments or as a herbal supplement for health promotion. It grows abundantly in south eastern Asia, i.e., India, Sri Lanka, Pakistan, Java, Malaysia and Indonesia but it is cultivated extensively in India, China and Thailand, the East and West Indies, and Mauritius [10]. It is an annual, branched, herbaceous plant erecting to a height of 30-110 cm in moist shady places with stem acutely quadrangular, much branched, easily broken, fragile texture stem. Leaves are simple, opposite, lanceolate, glabrous, 2-12cm long; 1-3cm wide. The flowers possess botanical features of calyx 5- particle, small, linear; corolla tube narrow, about 6 mm long; limb longer than the tube, bilabiate; upper lip oblong, white with yellowish top; lower lip broadly cunneate, 3-lobed, white with violet markings; stamens 2, inserted in the throat and far exserted; anther basally beared. Superior ovary, 2-celled; style far exserted. Seeds are very small, sub quadrate [9,11]. Based on this background, the present study was aimed to investigate the protective effect of *Andrographis paniculata* an hyperglycemic mediated oxidative damage in the renal tissues of experimental diabetic rats.

MATERIAL AND METHODS

Collection of plant material

The medicinal plant *Andrographis paniculata* were collected from Adhiparasakthi Agricultural College, medicinal garden, kalavai, vellore district, Tamil nadu, India. The plants were authentified by the herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, Chennai. A voucher specimen no: PARC/2015/ 3001.

Andrographis paniculata plants extract preparation

After the collection of *Andrographis paniculata* medicinal whole plant were placed in clean tray and allowed for shade drying. The plant were subjected to surface sterilization using ethanol and then dried in shade. The dried plant leafs were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh).

The *Andrographis paniculata* whole plant powdered (250 g) was defatted by treating with pet-ether and then extracted with ethanol solvent by using Soxlet apparatus. The solvent was removed under vacuum to get the solid mass. The residue was weighed and stored in air and water proof containers, kept in refrigerator at 4^{o}C. From this stock, fresh preparation was made whenever required [12].

Phytochemical examination

To investigate the phytochemical present in the *Andrographis paniculata* ethanol extract by using standard procedures [12].

GC - MS Analysis of bioactive compounds from *Andrographis paniculata*

The methanol extract obtained from sample was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. Some of the important features are summarized below. GC-MS analysis of the sample was carried out using Perkin Elmer Make clarus 680 with Elite-5MS (30.0 m, 0.25mmID, 250µm df) Column. Helium was used as the carrier gas and the temperature programming was set with Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min., 1 µL samples were injected with split less mode. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 32.00 min. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Animals

Healthy adult *wistar albino* rats (weighing 160 - 210g) were used in the experiments. Animals were housed in polypropylene cages at 22±2^{o}C with relative humidity of 45- 55% under 12 hour's light and dark cycle. They were feed with standard laboratory animal feed (Hindustan Lever Ltd., India) and water *ad libitum*. Ethical clearance was obtained from the Institutional Animal Ethical Committee - Approval No: IAEC/ APCAS/ 01/2015/06.

Acute toxicity study

The LD_{50} study was performed as per OECD – 423 guidelines for the medicinal plant *Andrographis paniculata* ethanol extract to find out maximum tolerable doses and minimum lethal doses producing 100% death. The formulation was given individually, by oral route through intragastric tube to the group of rats and the LD_{50} values were calculated [13].

Induction of diabetes mellitus

The experimental animal in this model were the male, adult Wistar albino rats, weighing 180- 210g. After a 12-hour fast, the rats were weighed and a solution of Streptozotocin in prepared in 0.1 M Citrate buffer at 4.5 pH in corresponding to 45 mg / kg body weight was administered intraperitoneally in a single dose. Food and water were given to the rats after 30 minutes of drug administration [14]. After two weeks, rats with blood glucose levels of 200-260mg/dl. were used for the study.

Experimental design

Experimental rats were divided into 5 groups as consist of 6 rats in each group.

- Group I – Serves as Control and received orally 0.5% Carboxy Methyl Cellulose (CMC 3ml each) up to 32 days.
- Group II – Serves as Diabetic Control.
- Group III – Received oral dose 200mg/kg *Andrographis paniculata* ethanol extract respectively for 32days.
- Group IV- Received oral dose 400mg/kg *Andrographis paniculata* ethanol extract respectively for 32 days.
- Group V - Serves as received oral dose of standard drug Glibenclamide (10 mg/ kg body wt.) for 32 day.

Streptozotocin at a dose of 45 gm/kg body weight through IP. were administered to all the animals in groups of II, III, IV & V groups [14].

Collection of blood and kidney tissues

Blood was collected by retro orbital puncture after 12 hrs fasting and 2 hrs after giving the extract in gum acacia for the estimation of plasma glucose. Auto analyzer and kit methods were used in this study to minimize sample requirement.

After 32nd days treatment, the animals were fasted for 12 hrs, anaesthetized by using ketamine (24mg/kg body weight, intramuscular injection), and sacrificed by decapitation. Blood was collected in tubes with EDTA for the estimation of Plasma Lipid Peroxide markers and Antioxidants. Tissues (kidney) were surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice cold containers.

Processing of blood and tissue samples

Blood was collected in fresh test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min. and Blood collected in a heparinized centrifuge tube and centrifuged at 2000 rpm for 10 min and the plasma was separated by aspiration.

Tissue homogenate preparation

Liver and kidney tissues (250mg) were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate were centrifuged at 1000 rpm for 10 min at 0º c in cold centrifuge. The supernatant was separated and used for various bio chemical estimations.

Statistical analysis

The statistical analysis was performed by ANOVA under one way classification followed by Bonferroni multiple comparison test, changes were considered significant at the P-value of < 0.05 and < 0.01 level of significance. The values were expressed as mean ± SD.

RESULT AND DISCUSSION

Diabetes mellitus is an metabolic disorder characterized with hyperglycemia and free radical production. In present medicine, their no satisfactory effective therapy is still available to cure diabetes mellitus. Many medicinal plants are claimed to possess antidiabetic and antioxidant activity. In practice, it is being progressively more recognized to be an alternative approach to modern medicine for more effective and safe [15]. Presently, oxidative stress is suggested as mechanism underlying diabetes and diabetic complications. This results from an imbalance between radical producing and radical scavenging system. In diabetes mellitus condition, protein glycation and glucose autoxidation may produce free radicals, which in turn catalyze lipid peroxidation.

Phytochemical screening

Preliminary Phytochemical Screening proves the presence of biologically active principles like Flavonoids Alkaloids, Saponins, Triterpenoids, Cardiac glycosides and Tannins in the *Andrographis paniculata* ethanol extract **(Table 1).**

Table 1: Preliminary phytochemical investigation in the *Andrographis paniculata*

Name of the Phytochemicals	Presence / Absent
Alkaloids	++
Triterpenoids	++
Glycosides	+
Saponins	+
Phenol	+
Phytosterol	++
Protein	+
Flavanoids	+
Tannins	++
Essential oils	++
Carbohydrate	+

+ Present; ++ More amount present; - Absent

GC- MS Analysis

The GC- MS analysis of ethanol extract revealed the presence of 11 different compounds namely the retention time, molecular weight, relative percentage of compounds present in *Andrographis paniculata* were recorded in the Table 2 & Fig.1.

Table 2: Phytocomponents in the ethanol extract of *Andrographis paniculata*

S. No.	Retention Time	Name of the Compound	Molecular Formula	M.W.	Peak Area (%)
1.	16.24	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	$C_{20}H_{40}O$	296	14.820
2.	18.95	PHYTOL	$C_{20}H_{40}O$	296	9.671
3.	24.40	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL-, (ALL-E)-	$C_{30}H_{50}$	410	2.807
4.	24.97	HEPTACOSANE	$C_{27}H_{56}$	380	3.990
5.	25.09	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXENE	$C_{15}H_{26}O$	222	10.549
6.	26.18	10,12-TRICOSADIYNOIC ACID, TRIMETHYLSILYL ESTER	$C_{26}H_{46}O_2Si$	418	4.125
7.	26.28	TETRATETRACONTANE	$C_{44}H_{90}$	618	18.732
8.	26.41	3BETA.-HYDROXYGUAIA-4(15),10(14),11(13)-TRIEN-6,12-OLIDE8-(.ALPHA.,.BETA.-DIHYDROXYBUTYRATE)	$C_{19}H_{24}O_7$	364	11.667
9.	26.67	DL-.ALPHA.-TOCOPHEROL	$C_{29}H_{50}O_2$	430	4.429
10.	27.82	PENTATRIACONTANE	$C_{35}H_{72}$	492	4.457
11.	28.40	BETA.-SITOSTEROL	$C_{29}H_{50}O$	414	7.731

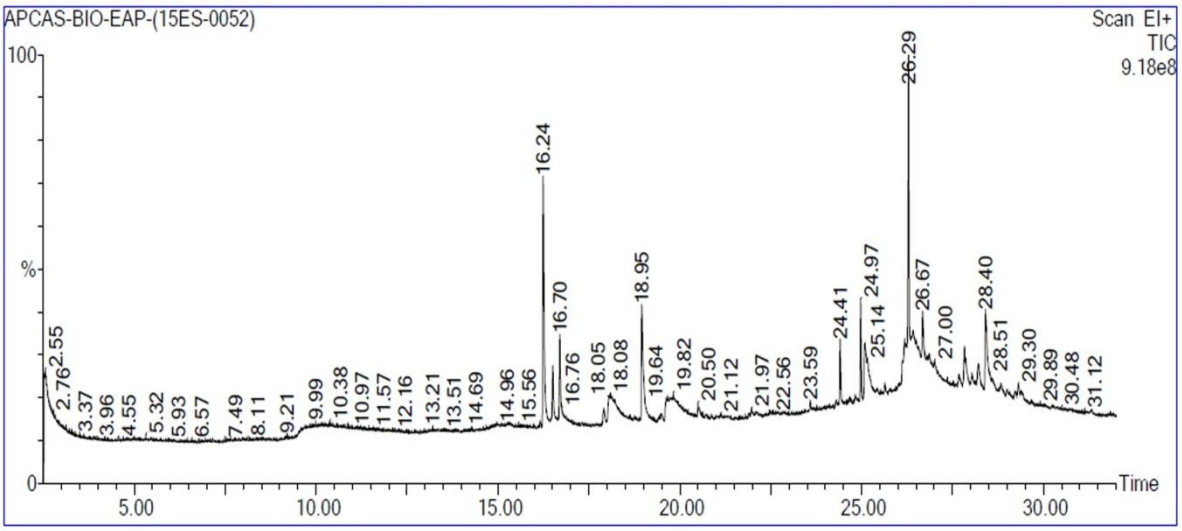

Figure 1: GC-MS Chromatogram of ethanol extract of *Andrographis paniculata*

Oral acute toxicity study

In acute toxicity study, *Andrographis paniculata* ethanol extract were found that the animal were safe up to a maximum dose of 3000mg/kg b.w. There were no changes in the normal behavioural pattern and no signs and symptoms of toxicity and mortality were observed.

Biochemical parameters analysis

Our results reword that ethanol extract of *Andrographis paniculata* (400 mg/kg of body weight) reduced the blood glucose and free radicals levels in diabetic rats when compared to normal rats. Table 3. Shows the levels of blood glucose increased in diabetic rats as compared to normal. The treatment of oral administration of *A. paniculata* ethanol extract in diabetic rats significantly decreased and brings back to near normal level when compared to diabetic rats. *A. paniculata* ethanol extract when compared to 200 mg/kg, the 400 mg/kg of body weight have the highly significantly (P< 0.01) reduce the blood glucose level.

Table 3: Effect of *Andrographis paniculata* ethanolic extract on blood glucose in normal and experimental diabetic rats.

Groups	blood glucose in initial (mg/dl)	blood glucose (mg/dl) in 32nd day
Normal	81.1 ± 0.4	82.4 ± 1.0
Diabetes	215.5 ± 0.5	226.4 ± 1.2*
Andrographis paniculata ethanolic extract (200mg/kg)	210.8 ± 0.5	102.7 ± 0.3**
Andrographis paniculata ethanolic extract (400mg/kg)	221.4 ± 1.3	106.6 ± 1.0**
Glibenclamide (10 mg/ kg body wt.)	222.2 ± 0.4	91.4 ± 0.0**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

During the experimental periods the changes in body weight were observed. The body weight found to be increased in normal and *A. paniculata* plant ethanol extract of treated diabetic rats and in diabetic rats it was decreased when compared with normal Table 4.

Table 4: Effect of *Andrographis paniculata* ethanol extract on animal body weight in normal and experimental diabetic rats..

Groups	Initial body weight (g)	body weight (g) 32nd day
Normal	182.3 ± 0.4	194.6 ± 0.2
Diabetes	181.4 ± 0.5	142.7 ± 0.3*****
Andrographis paniculata ethanolic extract (200mg/kg)	184.8 ± 0.5	191.6 ± 0.2******
Andrographis paniculata ethanolic extract (400mg/kg)	183.6 ± 1.3	192.6 ± 0.1******
Glibenclamide (10 mg/ kg body wt.)	184.3 ± 0.4	193.7 ± 0.2******

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

Administration of *A. paniculata* ethanol extract in diabetic rats exhibited considerable gain of body weight. During steady state treatment, when the rats administered *A. paniculata* ethanol extract had improved their glucose homeostasis to some extent, their food intake was similar and their growth rate was parallel. If suggests that *A. paniculata* extract has the anabolic action after initial adaptation to the treatment. Normalization of blood glucose by *A. paniculata* ethanol extract at 400mg/kg for 32 days treatment may be due to enhanced glucose transport across the cell membranes and glycogen synthesis and or glycolysis [16]. In this context thus are many number of other plants have been observed to have hypoglycaemic effect [17].

The changes in urine sugar during the experimental period were observed to find out the effectiveness of the methanol extract of *A. paniculata* plants. The excretion of urine sugar was found to be increased in diabetic rats when compared with normal *A. paniculata* plants ethanol extract treated diabetic rats was reverses back to the significantly normal condition, the absence of urine sugar in experimental rats. *A. paniculata* ethanol extract when compared to 200 mg/kg of body weight, the 400 mg/kg of body weight have the highly significantly (P< 0.01) increased the body weight and decreased urine sugar level experimental rats. Table: 5 shows the effect of *A. paniculata* extract on serum protein in normal, diabetic, *A. paniculata* plant treated diabetic rats. It was found that the level of serum protein was found to be decreased in streptozotocin induced diabetic rats when compared with normal. The decreased level of serum protein was bringing back to significantly (P<0.01) near normal in *A. paniculata* ethanol extract treated experimental diabetic rats.

Table 5: Effect of *Andrographis paniculata* ethanolic extract on serum protein in normal and experimental diabetic rats

Parameters	Normal	Diabetes	*Andrographis paniculata* ethanolic extract (200mg/kg)	*Andrographis paniculata* ethanolic extract (400mg/kg)	Glibenclamide
Serum protein (g/dl)	7.3 ± 0.23	6.2 ± 0.054**	6.8 ± 0.24*	6.9 ± 1.34**	7.0 ± 0.42**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

In diabetic rats, serum protein was decreased. Dehydration and loss of body weight have been associated with diabetic rats [18]. The decreased protein indicates the polyphagia condition and loss of body weight associated due to excessive breakdown of tissue protein and protein wasting due to unavailability of carbohydrates as an energy source. After the treatment of *A. paniculata* ethanol extracts these was significant improvement in the plasma protein.

The levels of serum cholesterol and blood urea were found to be elevated in streptozotocin induced diabetic rats when compared to normal. After treatment of *A. paniculata* extracts, it brings back to near normal levels of cholesterol and blood urea in experimental rats (Table: 6). The 400 mg/kg of body weight *A. paniculata* was significantly (P< 0.01) decreased in serum cholesterol and blood urea level when compared to 200 mg/kg of body weight.

Table 6: Effect of *Andrographis paniculata* ethanolic extract on cholesterol and blood urea in normal and experimental diabetic rats.

Parameters	Normal	Diabetes	*Andrographis paniculata* ethanolic extract (200mg/kg)	*Andrographis paniculata* ethanolic extract (400mg/kg)	Glibenclamide
Serum cholesterol (mg/dl)	162.0 ± 1.44	243.4 ± 0.24*****	176.3 ± 0.24*****	172.4 ± 0.24******	168.3 ± 1.30******
blood urea (mg/dl)	25.60 ± 1.04	49.03 ± 1.24*****	29.30 ± 0.20*****	28.23 ± 1.02******	26.60 ± 1.20******

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

In streptozotocin induced diabetic rats, the concentration of urea in liver was doubled when compared to normal. The increase may be due to the enhanced catabolism of both liver and plasma proteins. Almdal and Vilstrup (1988) have reported that insulin therapy in diabetes leads to normalization of organ nitrogen contents of urea synthesis. The cholesterol level of diabetic rats was also increased by two fold [19]. According to Feingola (1982), the *de nova* synthesis of cholesterol in the gut of diabetic rats in increased two fold when compared to other organs of the systems and this may be the reason for the increase in serum cholesterol level in diabetic rats.

Antioxidant activity of *Andrographis paniculata*

The levels of lipid peroxides in Plasma and kidney tissues was found to be elevated in streptozotocin induced diabetic rats when compared

to normal. After treatment of *A. paniculata* extracts, it brings back to near normal levels of plasma and kidney tissue lipid peroxides in experimental rats (Table 7).

Table 7: Effect of *Andrographis paniculata* ethanolic extract on lipid peroxides level in Plasma and kidney tissues of normal and experimental diabetic rats.

Parameters	Normal	Diabetes	*Andrographis paniculata* ethanolic extract (200mg/kg)	*Andrographis paniculata* ethanolic extract (400mg/kg)	Glibenclamide
Lipid peroxides (Plasma) (mmol/dl)	7.20 ± 0.46	23.10 ± 1.21*****	17.2 ± 0.23*****	12.4 ± 0.31******	12.0 ± 2.30******
Lipid peroxides (Kidney) (mmol/100g of Protein)	65 ± 2.06	127.10 ± 1.01*****	97.2 ± 0.43*****	89.4 ± 3.31******	92.0 ± 0.20******

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

Table: 8 & Fig. 2 shows the effect of *A. paniculata* extract on kidney tissue, Superoxide dismutase, Catalase and Glutathione peroxidase antioxidant enzymes in normal, diabetic, *A. paniculata* plant treated diabetic rats. It was found that the level of Superoxide dismutase, Catalase and Glutathione peroxidase were found to be decreased in streptozotocin induced diabetic rats when compared with normal. The decreased level of Superoxide dismutase, Catalase and Glutathione peroxidase were bringing back to significantly ($P<0.01$) near normal in *A. paniculata* ethanol extract treated experimental diabetic rats.

Numerous studies have demonstrated that oxidative stress is key pathogenic factor in the development of diabetic complications. Oxidative stress includes the production of highly reactive oxygen species that are toxic to the cell, particularly the cell membrane in which these radicals interact with the Lipid bi layer and produce Lipid Peroxides. However, Endogenous antioxidant Enzymes (Superoxide dismutase, Catalase and Glutathione Peroxidase) are responsible for the detoxification of deleterious oxygen species. The *A. paniculata* ethanol extract ability to scavenge free radicals.

Table 8: Effect of *Andrographis paniculata* ethanolic extract on Superoxide dismutase, Catalase, Glutathione peroxidase level in kidney tissues of normal and experimental diabetic rats.

Parameters	Normal	Diabetes	*Andrographis paniculata* ethanolic extract (200mg/kg)	*Andrographis paniculata* ethanolic extract (400mg/kg)	Glibenclamide
Superoxide dismutase (U/mg of Protein)	12.0 ± 1.44	6.4 ± 0.24*	8.3 ± 0.24*	9.4 ± 0.24**	9.3 ± 1.30**
Catalase (U/mg of Protein)	32 ± 4.04	22 ± 0.24*	29 ±4.24*	26 ± 1.21**	30 ± 1.00**
Glutathione peroxidise (U/mg of Protein)	9.0 ± 1.21	4.4 ± 0.31*	7.3 ± 1.04*	6.4 ± 2.04**	7.3 ± 0.20**

Values are expressed as mean ± SD for six animals in each group; *P < 0.05 and ** P < 0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

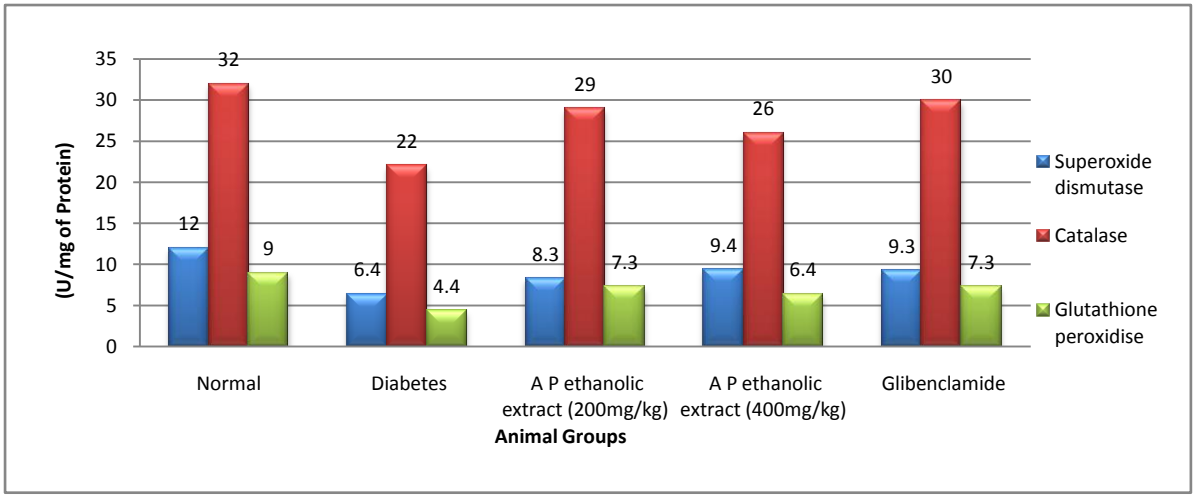

Figure 2: Effect of *Andrographis paniculata* ethanolic extract on Superoxide dismutase, Catalase, Glutathione peroxidase level in kidney tissues of normal and experimental diabetic rats.

SOD has been postulated as one of the most important enzyme in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen have diminishing the toxic effects caused by these radicals. Catalase (CAT) is a haemoprotien which catalyses the reduction of Hydrogen peroxides and protect the tissues from highly reactive hydroxyl radicals.

The Superoxide anion has been known to inactivate CAT, which is involved in the detoxification of Hydrogen Peroxide. GPx plays a primary role in minimizing oxidative damage. It has been proposed that GPx is responsible for the detoxification of H_2O_2 in low concentration, Where as Catalase come into play when GPx pathway is reaching saturation with the substrate. GSH metabolizing enzymes, Glutathione Peroxidase work in concert with Glutathione in the decomposition of H_2O_2 and other organic Hydro peroxides to non-toxic products, respectively, at the expense of reduced Glutathione.

The reduction in SOD and Catalase activities in Diabetic condition may be due to direct glycation of enzyme protein [20]. The increase in Superoxide radical in Diabetes may inhibit the activity of Catalase and Glutathione Peroxida. It has been concluded that the administration of *A. paniculata* ethanol extract may be attributed mainly due to the presence of Antioxidant phytochemicals.

Histopathological assessment of kidney cells

Induction of diabetes with streptozotocin was associated with marked histological changes in the kidney over a period of 32 days as revealed by tubular epithelial hypertrophy, glomerulosclerosis and glycogen accumulation. Treatment of diabetic rats with the *A. paniculata* ethanol extract afforded significant protection from renal damage whereas tubular damage was more pronounced in rats treated with glibenclamide (Fig. 3).

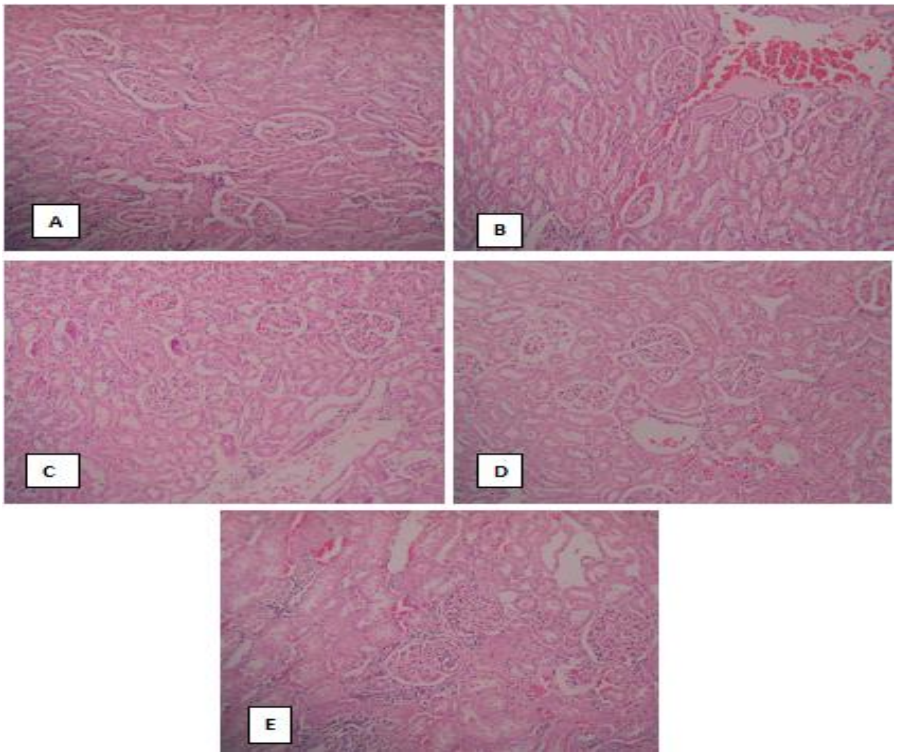

Figure 3: Photomicrographs of histopathological studies of Kidney sections of Normal and experimental diabetic rats. Paraffin embedded sections of renal cortex were stained with hematoxilin and eosin (H&E). Representative light micrographs (10 X) from each rat groups are shown. (HE-10X).

(A) Normal architecture of rat Kidney cells **(B)** Streptozotocin induced diabetic rats kidney cells **(C)** Streptozotocin + *Andrographis paniculata* ethanol extract (200mg/kg). **(D)** Streptozotocin + *Andrographis paniculata* ethanol extract (400mg/kg). **(E)** Streptozotocin + Standard drug treated (Glibenclamide).

The present findings demonstrated the *A. paniculata* ethanol extract have the protect hyperglycemic mediated oxidative damage in the renal tissues of experimental diabetic rats. According to traditional indigenous medicinal systems of India this plant has got several medicinal effects without producing any severe side effects. This plant could be very well used as prevention of hyperglycemic complications and tissues damaging through the oxidation.

CONCLUSION

In conclusion, *Andrographis paniculata* ethanol extract offers a promising therapeutic value in prevention of diabetes. These effects could be mainly attributed to its antioxidant properties as shown by significant quenching impact on the extent of lipid peroxidation, along with enhancement of antioxidant defense system in kidney tissues. Further studies will be needed in future to determine the main active ingredients having the beneficial antidiabetic and antioxidant effects.

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Conflicts of Interest

No conflicts of interest and there is no supporting grants/funding because of this is our own research project works.

Author's Contribution: Our investigator Dr. S. Rajesh Kumar is supporting the planning of research and writing manuscript.

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