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ANTIOXIDANT EFFECT OF *ALOE VERA* IN EXPERIMENTALLY INDUCED DIABETES MELLITUS

Jyoti Sethi*[1], Anupama Gupta [1], Sushma Sood [1], Kiran Dahiya [2], Gajender Singh [3] and Rajesh Gupta [4]

Department of Physiology [1], Department of Biochemistry [2], Department of Pharmacy [3], Department of Respiratory Medicine [4], Pt BD Sharma PGIMS, Rohtak, Haryana, India

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Correspondence to Author:

Dr. Jyoti Sethi

Associate Professor (Physiology), Pt B D Sharma PGIMS Rohtak- 124001, Haryana India

E-mail: dr_jyotisethi@rediffmail.com

ABSTRACT

The aim of the present study was to evaluate antidiabetic and antioxidant potential of *Aloe vera (AV)* in alloxan induced diabetes in albino rabbits. Experimental Diabetes was induced in rabbits with alloxan(80mg/Kg body weight)and animals showing fasting blood glucose levels more than 250mg/dl were considered as diabetics and divided into four groups of six each (n=6). Group I: Normal control rabbits, Group II: Alloxan induced diabetic rabbits, Group III: Diabetic rabbits received AV gel extract (300 mg/Kg) in aqueous solution for 21 days, Group IV: diabetic rabbits given glibenclamide (600ug/kg) in aqueous solution. All the drugs were administered orally (using an intra gastric tube) in a single dose in the morning for 21 days. Blood samples were collected from the marginal vein of pinna of overnight fasted rabbits (Blood sugar, glycosylated hemoglobin (HbA1c), Malondialdehyde (MDA), reduced glutathione (GSH), total thiols (PSH) and Superoxide dismutase (SOD). Oral administration of AV showed potent antihyperglycemic and anti-lipidperoxidative effect in diabetic animals. Simultaneously, the levels of protective antioxidant enzymes (SOD, GSH and PSH) were significantly increased with AV supplementation. The results suggest potent antidiabetic and antioxidant potential of AV in experimental diabetes, and thus *Aloe vera* can be used as an alternative remedy for treatment of diabetes mellitus and its complications.

INTRODUCTION: Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both [1]. It is associated with alterations in mitochondrial metabolism that result in both increased formation of reactive oxygen species (ROS) and failure of bioenergetics [2].

Free radicals and lipid peroxidation have been implicated in the aetiology of several major human ailments such as DM, cancer, cardiovascular diseases, arthritis and in aging. Oxidative stress can increase the rate of production of free radicals and hence induce lipid peroxidation [3]. The deleterious effects of free radicals are kept under check by a delicate balance between rate of their production and elimination by antioxidant defence mechanism and any shift in this delicate balance will lead to cellular damage. Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions [4].

Alloxan, is a chemical used for the induction of diabetes in animals, has been shown to damage pancreatic B cells by the liberation of oxygen radicals, with a reduction in antioxidant status [5].

World Health Organization (WHO) endorses the evaluation of the potential of plants as effective therapeutic agents, especially in areas where there is a lack of safe modern drug [6]. Oral hypoglycaemic drugs are used in the treatment of diabetes mellitus, but their use is limited by their pharmacokinetic properties, secondary failure rate and the accompanying side effects.

Medicinal herbs are an indispensible part of traditional medicine and India has a rich history of using various potent herbs and herbal components for treating diabetes, the most common being Bitter gourd (*Momorodica chorantia*), Neem (*Azadirachita indica*), Tulsi (*Ocimum sanctum*) and Garlic (*Allium sativum*) [7, 8, 9].

Aloe vera (synonym: *Aloe barbadensis* Miller) belongs to the Liliaceal family and is a cactus like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities [10]. There are some preliminary studies to suggest that oral administration of *Aloe vera* might be effective in reducing blood glucose levels in diabetic patients and in lowering blood lipid levels in Hyperlipidemia [11].

The present study was designed to investigate the protective effect of *Aloe vera* in lowering blood glucose levels, lipid peroxidation and antioxidant enzyme status in alloxan induced diabetic rabbits.

MATERIALS AND METHODS:

Animals: Albino rabbits of either sex weighing around 1.5-2.5 Kg were used in the study and were procured from disease free animal house of CCS Haryana Agriculture University, Hissar (Haryana, India). They had free access to food and water ad libitum and were maintained under 12:12 Hour light and dark cycles. Institutional Animal Ethical Committee (IAEC) approved the experimental protocol (vide Number/Phy/09/413 dated13.5.09) and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Plant material: Specimens of *Aloe vera* were collected from Arjun Park, Kurukshetra and were planted and cultivated in the greenhouse of the faculty of herbal garden of college of Pharmacy, PGIMS, Rohtak. Fresh leaves of this cultivated plant were used in the study.

Preparation of the extract: Mature, healthy and fresh leaves of *Aloe vera* having a length of about 75 to 90 cm were washed with fresh water. They were cut transversely into pieces. The thick epidermis of leaf was selectively removed and the semi solid gel in the centre was homogenized. The resulting mucilaginous, thick and straw colour homogenate was lyophilized using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in rotary evaporator.

The residue was stored in dry sterilized small containers at 4°C until further used. An aqueous suspension, which is the form customarily used in folk medicine, was prepared by dissolving suitable amount of ethanol free extract of *Aloe vera* leaf gel to get the desired concentration. The drug solution was prepared fresh each time and administered by intra gastric route. The dosing schedule was once per day [9].

Induction of experimental diabetes: Experimental diabetes was induced in rabbits with alloxan (80mg/Kg body weight) dissolved in 0.1M citrate buffer (pH-4.0) and injected intravenously to overnight fasted animals through their marginal ear vein [12]. Animals showing fasting blood glucose levels more than 250mg/dl were considered as diabetics and included in the study.

Experimental Design: The rabbits were divided into four groups of six animals (n=6) in each group as follows:

- Group I: Normal control rabbits
- Group II: Alloxan induced diabetic rabbits
- Group III: Diabetic rabbits received *Aloe vera* leaf gel extract (300 mg/Kg) in aqueous solution for 21 days.
- Group IV: Diabetic rabbits given glibenclamide (600ug/kg) in aqueous solution

All the drugs were administered orally (using an intra gastric tube) in a single dose in the morning for 21 days [13].

Sample collection: Blood samples were collected from the marginal vein of pinna of overnight fasted rabbits at the end of the experimental period. Fasting blood glucose (FBG), Glycosylated Haemoglobin (HbA1c),

Malondialdehyde (MDA) and antioxidant parameters- GSH (Reduced glutathione), Total Thiols (PSH), Superoxide dismutase (SOD), were determined at the end of the study. Final body weight of all rabbits was recorded. The serum was separated immediately and assayed for the following parameters.

1) Blood Glucose: By Glucose oxidase peroxidase method.[14]

2) HbA1c assay was done on auto analyzer using kits by Randox [15].

3) Malondialdehyde (MDA): The lipid peroxidation products reacted with thiobarbituric acid (TBA) to give a red chromogen, which was measured at 535nm spectrophotometrically [16].

4) Reduced Glutathione (GSH): Deproteinised serum was precipitated with metaphosphoric acid and was made to react with 5, 5'dithiobis-2-nitrobenzoic acid (DTNB) to produce a yellow chromogen, and absorbance was measured at 420nm against blank [17].

5) Superoxide dismutase (SOD): Epinephrine can be auto oxidised to adrenochrome by superoxide radicals. Maximum auto oxidation occurs at pH 10.2, which has been used as the basis for the assays of this enzyme [18].

6) Total Thiols (PSH):5, 5'dithiobis-2-nitrobenzoic acid reacts with total sulphydryl groups to form a chromogen whose extinction is measured spectrophotometrically at 420nm [19].

Statistical Analysis: The results were expressed as mean± SEM and were analysed using student t test (SPSS-14) and p value <0.05 was considered significant.

RESULTS: Blood glucose levels in alloxan induced diabetic rabbits (Gp-II) were significantly ($p<0.05$) elevated as compared with control rabbits (Gp-I). Blood glucose levels also showed a reversal near to control levels after treatment with glibenclamide and *Aloe vera* (**Table 1**).

Lipid peroxidation (as measured by MDA) and antioxidant levels are shown in **Table 2**. The results indicate that lipids of diabetic rabbits are vulnerable to peroxidation due to increased oxidative stress ($p<0.01$). Treatment with glibenclamide and *Aloe vera* was able to reverse the altered lipid peroxidation damage as indicated by MDA levels (Table 2).

Antioxidant levels (GSH, PSH and SOD) in diabetic rabbits were significantly reduced as compared to control group. Supplementation of *Aloe vera* nearly normalized the levels of these antioxidant enzymes.

TABLE 1: SHOWING VALUES OF FASTING BLOOD GLUCOSE, GLYCOSYLATED HAEMOGLOBIN AND BODY WEIGHT N VARIOUS GROUPS

Group & treatment	Blood glucose (mg/dl)	Glycosylated haemoglobin (%Hb)	Body weight (kg)
Gp-I (N. control)	150 ±16.08	3.01 ±0.10	1.90 +0.04
Gp-II(DM control)	266.17 ±14.08 a	6.40 ±0.12 a	1.52 +0.05 a
Gp-III(DM+ *Aloe vera*)	182 ±12.26 b	5.20 ±0.18 b	1.72 +0.06 b
Gp-IV (DM + glibenclamide)	160 +13.84 b	5.80 ±0.19 b	1.84 +0.06 b

TABLE 2: SHOWING ANTIOXIDANT LEVELS IN VARIOUS GROUPS

Group & treatment	Plasma MDA (nmol/ml)	Whole Blood Glutathione (mmol/l)	Whole Blood Total Thiols (mmol/l)	Plasma SOD (EU/ml)
Gp-I (N. control)	3.28±0.46	0.92±0.12	2.96±0.26	3.70±0.80
Gp-II (DM control)	5.63±1.06 a	0.48±0.18 a	1.90±0.30 a	2.18±0.78 a
Gp-III (DM+ AV)	4.28±0.60 b	0.61±0.13b	2.14±0.38 b	2.96±0.80 b
Gp-V (DM+glibenclamide)	4.00±0.80 b	0.68±0.16 b	2.28±0.32 b	3.12±0.76 b

a; $p<0.001$ as compared to Gp I, b; $p<0.01$ as compared to Gp II

DISCUSSION: Diabetes has been found to be associated with indices of oxidative damage. In diabetes, hyperglycaemia generates reactive oxygen species (ROS) which in turn causes lipid peroxidation and membrane damage and plays an important role in the pathogenesis of secondary complications of diabetes [20]. Antioxidants can provide defence against free radical damage. Diabetic complications can be

prevented or retarded by administration of appropriate antioxidants, in addition to traditional therapeutic principles [21]. Alloxan mediated damage of pancreatic cells is mediated by ROS. Alloxan and the product of its reduction dialuric acid establish a redox cycle with the formation of superoxide radicals, which undergo dismutation to form hydrogen peroxide. Dialuric acid has been observed to stimulate lipid peroxidation *in vitro* [22].

In the present study, increased MDA level in alloxan induced diabetic rabbits suggests increased lipid peroxidation. Oral administration of *Aloe vera* significantly decreased ($p<0.01$) the levels of MDA in group IV animals. Herbs like *Piper nigrum* and *Vinca rosea* have also been reported to reverse the altered lipid peroxidation damage in alloxan induced diabetic rats [23].

Glycated haemoglobin (HbA1c) is being used with increasing frequency to monitor long term blood glucose control in Diabetes mellitus. The non enzymatic reaction of glucose with proteins is widely recognized and is thought to be an important component in the aetiology of long term complications of Diabetes mellitus. This non enzymatic modification of proteins alters not only the structure, but also the biological properties of proteins [24].

In our study, the higher levels of HbA1c in gp.-II(diabetic rabbits)as compared to gp.-I (control) indicate poor glycemic control. Treatment with Aloe vera (gp.-III) led to significant decrease in HbA1c levels, suggesting improved glycemic status. Ayesha Noor *et al*., have observed that *Aloe vera* extract has significant hypoglycaemic activity though the exact mechanism is still speculated [25]. There is evidence indicating that glycation reaction can be modulated by the levels of reduced glutathione and MDA [26, 27].

Total thiols play a vital role in the structure, activity and transport function of proteins, membranes and enzymes and have been found to decrease the damage provoked by oxidative stress [28]. Glutathione accounts for 90% of non-protein thiols in the cell, and it acts as primary line of defence to cope with deleterious effects of ROS.[29] Decreased levels of glutathione in diabetic animals (gp-II) indicates increased susceptibility to oxidative stress.

There are reports of decreased levels of liver and kidney GSH in alloxan induced diabetes [30]. Supplementation of *Aloe vera* in group III tried to increase the levels of GSH, probably by facilitating the reduction of free radicals by H^+ donation. *Aloe vera* probably improved the glucose uptake by the cell and therefore exerted a hypoglycaemic effect. The increased glucose uptake by cell via HMP shunt probably led to increased production of NADPH+H+ and thus tried to normalise GSH levels in group IV.

SOD detoxifies superoxide free radicals and converts them to H202, which is further converted to H2O by catalase or GSH peroxidase. There are reports of both increased [31], decreased [32] as well as unchanged [33] SOD activity in diabetic animals .In our study, the activity of SOD was lower in diabetic animals. Administration of Aloe vera brought back the decreased levels to normal indicating better antioxidant status.

The result of the present study indicate that administration of AV improved the antioxidant status as well as brought about significant reduction in MDA levels suggesting that AV probably can modulate cellular antioxidant levels. Since it is cheap, readily available to all strata of society, with medicinal properties attributed to it, further studies are needed to elucidate the active principle, so that its use among general population can be promoted.

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