IJP (2016), Vol. 3, Issue 7 **(Research Article)**

Received on 22 June 2016; received in revised form, 18 July 2016; accepted, 29 July 2016; published 31 July 2016

ANTI-DIABETIC AND ANTI-OXIDANT PROPERTIES OF *ALOE VERA* IN ALLOXAN INDUCED DIABETIC RATS

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Keywords:

A. vera, Diabetes, Alloxan, Antioxidant, Free radical

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ABSTRACT: The present study was undertaken to evaluate antidiabetic and antioxidant activities of *Aloe vera* (*A. vera,* Ghee-kuwar; family: Liliaceae) extract against alloxan-induced diabetes in male adult Charles foster rats to scientifically validate its use against diabetes. *A. vera* extract and a standard drug (glibenclamide) prepared in aqueous gum acacia (2%, w/v) suspension and fed orally to alloxan induced diabetic rats for 30 days. Biochemical parameters in normal, diabetic control, standard (600 µg/kg bw p.o.) and treated (1 g/kg bw p.o.) animals group were determined and compared. Treatment of alloxan-induced diabetic rats with *A. vera* extract showed a significant reduction ($p<0.001$) in blood glucose, lipid peroxide and significantly increased ($p<0.001$) superoxide dismutase, catalase, and protein. Furthermore, the extract (100-200 µg) when tested for its antioxidant activity *in-vitro*, showed significant ($p<0.001$) inhibition in the generation of superoxide anions and hydroxyl radicals. The results of the present study demonstrated anti-diabetic and anti-oxidant activities of *A. vera* extract which could help in the treatment of diabetes and related complications.

INTRODUCTION: Diabetes mellitus is as old as mankind, and its incidence is considered to be high all over the world [1]. In ancient India, diabetes was also known as "Madhumeha" [2].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.3(7).319-24

Article can be accessed online on:
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(7).319-24

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia with glycosuria, and it is well documented that increased level of blood glucose is a marker of disorder of carbohydrate metabolism and is associated with the initiation of diabetic dyslipoproteinemia and other complications [3]. DM remains a major health problem, and its prevalence is increasing day by day. There are an estimated 150 million people around the globe who have diabetes, almost five times more than the estimates ten years ago and the

figure is expected to reach 300 million by the year 2025. [4] The recent most figure is about 180 million diabetics worldwide, and 5% annual death is due to diabetes [5]. Prevalence of diabetes in India has the maximum increase in the last few years, ten years ago, prevalence of diabetes in India was 2.4% in rural and 8.2% in urban areas [6]. According to a survey, approximately 60 million people were reported diabetic in India [7].

Aloe vera has been used for medicinal purposes in several cultures for millennia: Greece, Egypt, India, Mexico, Japan, and China. *Aloe vera* is a member of the Liliaceae family, which has about 360 species. *Aloe vera* is a cactus-like plant that grows in hot, dry climate [8]. It is cultivated in large quantities. Cosmetic and some medicinal products are made from the mucilaginous tissue in the center of the leaf, known as *Aloe vera* gel. The peripheral bundle sheath cells of *Aloe vera* produce an intensely bitter, yellow latex, commonly called aloe juice, or sap [9]. The pharmacological actions of *Aloe vera*, as evidenced by *in-vitro* and animal studies, include anti-inflammatory and anti-arthritic activity, and antibacterial and hypoglycemic effects. The therapeutic claims made for *Aloe vera* range over a broad list, as do the pharmacological activities associated with it [10]. Most of these claims are based on historical use rather than hard evidence.

Free radicals are species capable of independent existence containing one or more unpaired electron(s) which makes them para-magnetic and highly reactive. The formation and scavenging of free radicals and other oxygen-derived species in the biological system have received much attention. Free radicals are reported to play an essential role in various human diseases, such as Alzheimer's disease, male infertility, diabetes, atherosclerosis, Parkinson's, *etc*. [11, 12] There is the number of reports implicating these highly reactive oxygen products in the pathogenesis of disease [13].

Moreover, there are also reports that the disturbed anti-oxidant levels may be due to the acceleration of some cellular reactions or insufficiency of the antioxidant defense system [14]. During the last few years, alternative medicine has been practiced as an effective tool for therapeutic purposes [15, 16]. Among alternative medicine methods, herbal remedies are now widely accepted therapy [17]. Recent studies from our laboratory have revealed the repair of oxidative stressed state using anti-oxidants [18], and the control of this stress using herbal preparations [19]. In the present study, we have tried to assess *Aloe vera* extract for an anti-oxidant property. This study will help in understanding the mechanism of antioxidant potential of *Aloe vera* and open up new avenues for treatment of human diseases linked to oxidative stress.

MATERIAL AND METHODS:

Preparation of *Aloe vera* Extract: *Aloe vera* were collected from the local area of Lucknow and identified taxonomically by Department of Pharmacology, Era's Lucknow Medical College, Lucknow. A voucher specimen (AV-005/10) was also submitted. The aqueous extract of *A. vera* was prepared by boiling 50 gm of the plant leaf gel with 100 ml of water for 10 min. After cooling to room temperature, the extract was filtered and stored in the refrigerator until used. The dose (1g/kg, b.w.) was administered orally daily for 30 days.

Animals: *In-vivo* experiments were conducted as per CPCSEA guidelines provided by the Animal Ethics Committee of Institute. Male adult rats of Charles Foster strain (200-225g) were used in the study. The study was conducted after the approval of the Institutional animal ethics committee (IAEC/PV/08/14). The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26°C, relative humidity 50-60% and 12/12 h light/dark cycle (light from 8:00 am to 8:00 pm) and provided with standard rat pellet diet and water *ad libitum*.

Alloxan-Induced Hyperglycemia: Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate 150 mg/kg b.w. After two weeks of diabetes induction, rats with serum glucose level 280-367 mg/dl were taken for the study.

Experimental Design: The rats were divided into four groups having six animals in each as follows:

Group 1: Control rats (on normal saline);

Group 2: Alloxan-induced diabetic rats (on normal saline);

Group 3: Alloxan treated diabetic rats + *A. Vera* extract (500 mg/kg b.w);

Group 4: Alloxan treated diabetic rats + glibenclamide (600 µg/kg b.w). After 30 days, rats were fasted overnight, anesthetized with thiopental solution, and injected (i.p.) with 1 ml/kg b.w. of 10 mg/ml solution of heparin. After 30 days, blood was drawn from the retro-orbital plexus and collected in EDTA coated tubes. The blood was used for the estimation of glucose; simultaneously plasma was separated and used for the estimation of antioxidant parameters.

Estimation of Antioxidant Activity:

Generation of Superoxide Anions: The effect of *A. vera* extract on the generation of superoxide anions (O_2^-) *in-vitro* was investigated in a reaction system comprising of phenazine methosulphate, NADH and NBT [20]. After 90 sec incubation in absence or presence of test extract at concentrations 100 and 200 µg, the amount of formazone formed was read at 560 nm against respective reagent blank. Standard used was Allopurinol.

Generation of Hydroxyl Radical: *A. vera* extract (100 and 200 µg) was tested against the formation of hydroxyl radicals (OH^-) *in-vitro* in a reaction system composed of $FeSO_4$, sodium ascorbate, H_2O_2, and deoxyribose. After reaction in the absence or presence of root extract, incubation mixture was assayed on the spectrophotometer at 510 nm for malondialdehyde formed [21]. Standard used was mannitol.

Blood Glucose and Protein Analysis: Blood glucose and protein estimation were done using the Kit method, and the technical bulletin supplied with the kit was followed.

Biochemical Analysis of Liver: Liver was homogenized (10%w/v) in cold 1 M phosphate buffer (pH 7.2). Liver homogenate 10%w/v in 0.15 M KCl was used for the estimation of superoxide dismutase (SOD) [20] and catalase (CAT) [22].

Statistical Analysis: One-way analysis of variance (ANOVA) was performed with values obtained for different study groups. All hypothesis testing were two-tailed. $P<0.05$ was considered statistically significant, and the results were expressed as mean ± SD. The Graph pad INSTAT 3.0 software was used to carry out the statistical analysis [23]. The generation of free radicals with different concentrations of *A. vera* extract was compared with that of their formation without extract. The values were tested for significance at $P<0.05$.

RESULTS:

Effect of *A. vera* Extract in Alloxan-Induced Hyperglycemia: The acute administration of alloxan caused a marked increase in their plasma levels of blood glucose 273%. The hypoglycemic activity of *A. vera* extract was comparatively less to that of glibenclamide **Table 1**.

Effect of *A. vera* Extract on Lipid Peroxidation: Our results show a significant increase (246%) in lipid peroxides in alloxan-induced diabetic animals as compared to normal mice. On the other hand, *A. vera* extract treated animals showed the reduced formation of lipid peroxides (62%), which was comparable to standard drug glibenclamide treated animals.

Effect of *A. vera* Extract on Hepatic SOD, CAT, and Protein in Alloxan-Induced Diabetic Rats: Our data show that administration of alloxan in rats decreases the levels of SOD 26%, CAT 28%, and protein 28% respectively. Treatment with *A. vera* extract reactivated SOD 28%, CAT 21%, and protein by 27%. Glibenclamide increased the activity of SOD 28%, CAT 35%, and protein levels by 30% respectively) **Table 1**.

Effect of *A. vera* Extract on Generation of Superoxide Anions: Our investigations show that *A. vera* extract trapped the O_2^- anions generated by the non-enzymic system of NADH–phenazine–methosulphate and were responsible for the reduction of NBT in the reaction mixture. The effect was dose-dependent and was highest by 24% at 200 µg/ml of test extract.

Effect of *A. vera* on Generation of Hydroxyl Radicals: Our investigations also showed that *A. vera* extract when added with a reaction mixture containing Fe^{2+}–sodium ascorbate–H_2O_2 employed for non-enzymic generation of OH^- inhibited fragmentation of deoxyribose into MDA and this effect was maximum by 34% at peak concentration (200 µg/ml) of test extract.

TABLE 1: EFFECT OF *A. VERA* ON SERUM LIPID PEROXIDE, HEPATIC SOD, HEPATIC CATALASE AND PROTEINS IN ALLOXAN INDUCED DIABETIC RATS

Animal group	Plasma glucose (mg/dl)	Lipid peroxide (μmol MDA/ml)	Hepatic SOD (μmol/min/mg protein)	Hepatic CAT (unit/mg protein)	Protein (g/dl)
Control	91.22±7.31	3.80 ±0.30	3.42 ± 0.34	39.00 ± 30.12	7.22±0.37
Alloxan induced diabetic	340.48±22.60*** (+373%)	9.37 ± 0.47*** (+260%)	2.50 ± 0.21*** (-20%)	28.00 ± 21.39*** (-28%)	5.20±0.18*** (-27%)
Alloxan induced diabetic + *A. vera*	256.27±21.93 (-25%)	5.88 ± 0.17*** (-37%)	3.21 ± 0.17* (+22%)	33.89 ± 27.77* (+17%)	6.60±0.10** (+21%)
Alloxan induced diabetic + Glibenclamide	238.77±21.14*** (-30%)	5.82 ± 0.37*** (-37%)	3.22 ± 0.33** (+22%)	37.99 ± 27.11*** (+26%)	6.80±0.27 (+24%)

Values are expressed as mean ± SD of six rats. Alloxan induced diabetic group is compared with the control and drug-treated group. Values in parenthesis indicate percent change. *P<0.05; **P<0.01; ***P<0.001

TABLE 2: EFFECT OF *A. VERA* EXTRACT ON GENERATION OF OXYGEN FREE RADICALS *IN-VITRO*

Experimental Schedule	Dose μg/ml	Superoxide anions (n mole formazone formed/minute)	Hydroxyl radicals (n mol MDA/hr)
Control		169.92 ± 13.14	92.62 ± 5.87
A. vera	100	148.19 ± 10.18* (-13%)	72.35 ± 7.00** (-22%)
	200	129.43 ± 8.77*** (-24%)	60.80 ± 5.21*** (-34%)
Standard drug	200	55.12 ± 3.82*** (-68%)	35.32 ± 3.00 (-62%)
		Allopurinol	Mannitol

Values are expressed as mean ± SD of six separate observations. The system added with *A. vera* extract was compared with those without adding the extract. *P<0.05, **P<0.01, ***P<0.001

DISCUSSION: In the present study, *A. vera* was tested for its anti-diabetic and anti-oxidant activities in alloxan-induced diabetic rats. Alloxan causes reversible damage to insulin-producing b-cells found in the pancreas, and that is why this animal model has been used for primary screening of test drugs for antidiabetic activity [24]. We found that intoxication with alloxan caused increased levels of plasma glucose in rats and their reversal by the treatment with *A. vera* extract.

Furthermore, extract also reduced lipid peroxide levels in diabetic rats following inhibition of ROS generation *in-vitro*. The phytochemical studies showed that *A. vera* contains a variety of sterols, carbohydrates, glycosides, tannins, and flavonoids [25]. It is suggested that all or some of these bioactive compounds may be responsible for hypoglycemic and antioxidant effects of this herb. The *Aloe* parenchyma tissue or pulp has been shown to contain proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds and small organic compounds in addition to the different carbohydrates [26].

Chandan and workers demonstrated the therapeutic efficacy of the water extract of *A. vera* against carbon tetrachloride-induced liver damage as indicated by reversal of centrilobular necrosis, macro-vascular fatty changes and scattered lymphomononuclear cell infiltrate in hepatic parenchyma [27]. In another study, *A. vera* has been shown that high concentrations are required to achieve modest activation of macrophages as compared to crude *A. vera* juice, which suggested that there is an effective component in the juice responsible for the macrophage activation [28].

However, there are few pharmacological investigations available on this plant done so far. The present work is a report on new properties of *A. vera* to exert hypoglycemic as well as antioxidant activities *in-vivo* and *in-vitro* models. Further work on drug metabolism and to assess the biological activity of *A. vera* extract is under progress to substantiate the present findings.

CONCLUSION: The outcomes of the present study suggest that the extracts of *A. vera* can

contribute their potential as anti-diabetic and antioxidant drugs to the world of natural products in the field of dyslipoproteinemia. It should be pointed out here that plant-derived natural compounds have established a proven platform for developing new drug synthesis with fewer side effects. Our study validates a strong antioxidant and anti-diabetic activities of *Aloe vera* in hyperglycemic rats.

ACKNOWLEDGEMENT: One of us (Prabha Verma) is grateful to the Director, Central Drug Research Institute, Lucknow and Era's Lucknow Medical College & Hospital, Lucknow for experimental and financial support.

CONFLICT OF INTEREST STATEMENT: The authors declare that they have no conflict of interest.

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How to cite this article:
Verma P, Kumar V, Rathore B, Singh RK and Mahdi AA: Anti-diabetic and anti-oxidant properties of *Aloe vera* in alloxan induced diabetic rats. Int J Pharmacognosy 2016; 3(7): 319-24. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(7).319-24.