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Original Research

Ameliorative effects of aqueous leaf extract of aloe arborescens on anti- hyperglycaemia and anti-hyperlipidemia alterations in alloxan-induced diabetic mice

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Abstract

Diabetes poses great challenge to the world's health care system. Its worldwide prevalence was estimated at 366 million in 2011 of these, 183 million people were believed to be unaware of their condition. If no measures taken, the prevalence is projected to rise to 552 million people by 2030, representing around 10% of the global adult population. The aim of present study was designed to examine the potential anti-hyperlipidaemic and anti-hyperlipidemia efficacy of the aqueous extract from *Aloe vera* leaf gel in alloxan-induced diabetic mice. The animals were divided into three groups of normal mice; alloxan-induced diabetic mice and diabetic mice treated with 300mg/kg b.w of the aqueous leaf extract, respectively. Treatment was *via* the oral route for 21days. Various biochemical parameters, including lipid profile and serum glucose were decreased as well as HDL-C increased. Statistical analysis were done by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. These results demonstrate the anti-diabetic and anti-hyperlipidaemic activities of *Aloe vera* leaf extract in diabetic mice.

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INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system. Worldwide, the number of people with diabetes is expected to double over the 13 year period from 1997 to 2010, so that it is expected that there will be over 221 million people with diabetes worldwide by 2010 [1]. Diabetes is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism [2]. These traits are hypothesized to be responsible for the damage to cell membranes, which, in turn, results in an elevated production of reactive oxygen species (ROS) [3]. The elevated generation of ROS and the simultaneous decline in antioxidative defence mechanisms observed in diabetic patients could promote the development of late complications [4]. To reduce the risk of late complications and negative outcomes of DM , such as blindness, renal failure and limb amputation, the control not only of blood glucose levels, but also lipid levels is necessary [5]. From the beginning of the last century, evidence of the lipid lowering properties of medicinal plants has accumulated [6]. Many scientists have demonstrated the role of medicinal plants in the

control of hyperlipidemia. Ethno botanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [7], but only a few have received scientific scrutiny. In this context, *Aloe vera* can rightly be mentioned as a plant of considerable interest.

Aloe vera 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 [8]. 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 [9].

Alloxan is a toxic glucose analogue, which selectively destroys insulin producing β-cells in the pancreas. When administered to rodents and many other animal species, it causes insulin dependent DM (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 DM in humans. Alloxan is selectively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in beta cells through uptake via the GLUT-2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The β-cell toxic action of alloxan is initiated by free radicals in this redox reaction [10].

MATARIALS AND METHODS

Animals

Healthy Swiss albino mice of both sexes, weighing approximately (28 g to 32 g) were used in the pharmacological studies. Before and during the experiment the animals were maintained in well-ventilated room at room temperature with natural day-night cycle in polypropylene cages lined with husk in standard environmental conditions (temperature 22±2 ºC, relative humidity 55±10% and 12:12 light:dark cycle). The mouse was fed on a standard pellet diet *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the (CPCSEA Regd. No. 1129/bc/07/ CPCSEA, dated 13/02/2008).

Chemicals

All chemicals were obtained from the following sources: alloxan was purchased from the Loba chemie (Batch no-G204207), Mumbai. Commercially available kits for chemical analyses such as glucose, cholesterol, triglycerides and HDL-cholesterol were used with crest coral clinical system, Goa, India. Analytical grade ethanol was purchased from Merck Company.

Preparation of plant material

The fresh leaves of *Aloe vera* were procured from local garden (Allahabad, U.P). The identity of the leaves of *Aloe vera* was confirmed by Botanist, Department of Botany, SHIATS, Allahabad, UP India. The leaves were washed with distilled water and dried completely under the mild sun and crushed with electrical grinder coarse powder. Aqueous extract was made by dissolving it in distilled water using by mortar and pestle. The dose was finally made to 300 mg/kg body weight for oral administration after the LD_{50} estimation.

Induction of hyperglycemia with alloxan

The selected mice were weighed, marked for individual identification and fast for overnight. The alloxan (dissolve in distilled water) at the rate of 150 mg/kg body weight [11] were administered intraperitoneal (i.p) for making the alloxan induced diabetic mice model. Hyperglycemia was confirmed by elevated blood glucose level, determined at 3rd day post-induction. The entire mice became consistently hyperglycemia and stable by after 7th day post-induction. Mice showing fasting blood glucose level above 200 mg/dl were selected for the study.

Experimental procedure

Mice were divided into three groups, with six mice in each group, as follows:

(i) group I, control mice; (ii) group II, alloxan-induced diabetic control mice; (iii) group III, diabetic mice given *Aloe vera* leaf extract (300 mg/kg) in aqueous solution daily for 21 days. After the study period, the animals were kept overnight fast and sacrificed. Blood samples were collected by orbital sinus puncture method [12] and centrifuged at 3000 rpm for 15 min. Serum was separated and analyzed for various biochemical estimations. Glucose by Trinder P. 1969 [13], cholesterol [14], triglycerides [15], HDL-cholesterol [16] and VLDL-cholesterol was calculated as triglycerides/5. LDL-cholesterol was calculated by the equation (Freidewald's Formula) [17]: LDL-cholesterol = Total serum cholesterol – (HDL + VLDL).

Statistical analysis

Results were presented as mean ± S.D. and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among means had been analysed by applying Tukey's multiple comparison test at 95% (p<0.05) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

RESULTS

The serum glucose levels showed elevation in the levels of the alloxan induced diabetic group in comparison to control group while the *Aloe vera* showed the glucose lowering down activity denote the antidiabetic effect. The lipid profile shows elevation in the levels of Total cholesterol , TG, LDL-C ,VLDL-C, levels in alloxan induced groups but decreased value of HDL-C while the *Aloe vera* showed declination in the Lipid profile levels and increased HDL-C levels denote the anti-hyperlipidaemic effects (Figures 1-6).

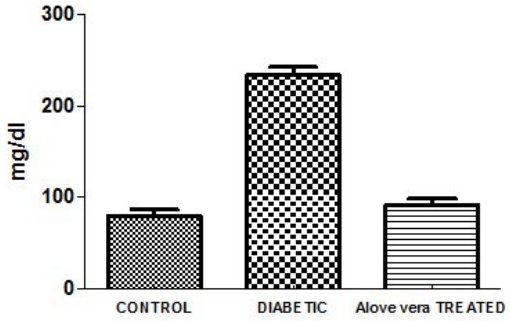

Fig 1. Effect of *Aloe vera* on diabetic induced group showing serum glucose level (n=6, values are mean ± S.D).

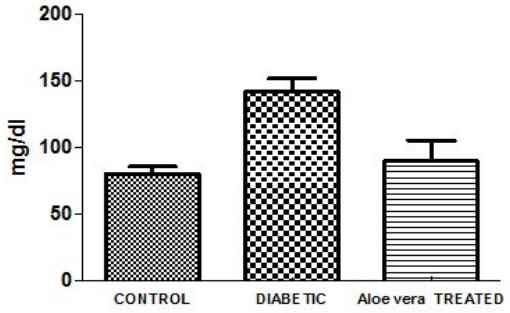

Fig 2. Effect of *Aloe vera* on diabetic induced group showing Total cholesterol level (n=6, values are mean ± S.D).

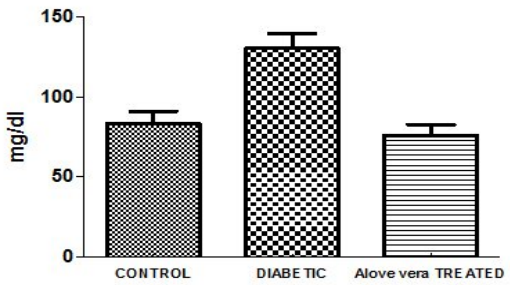

Fig 3. Effect of *Aloe vera* on diabetic induced group showing TG level (n=6, values are mean ± S.D).

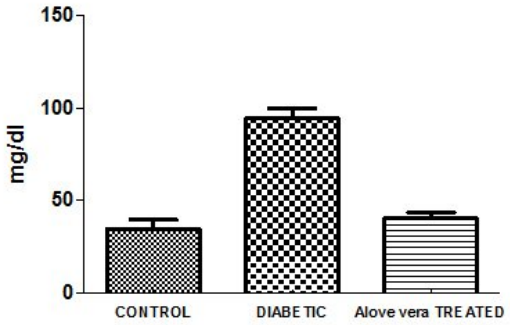

Fig 4. Effect of *Aloe vera* on diabetic induced group showing LDL-C level (n=6, values are mean ± S.D).

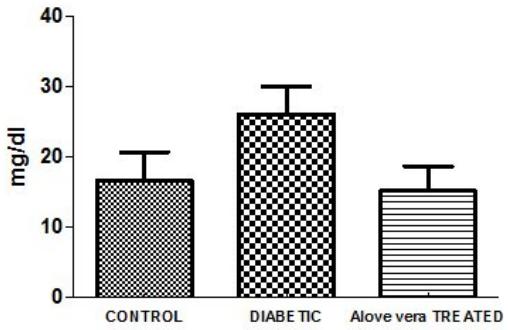

Fig. 5. Effect of *Aloe vera* on diabetic induced group showing VLDL-C level (n=6, values are mean ± S.D).

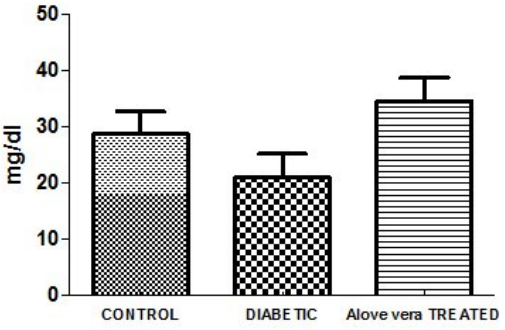

Fig 6. Effect of *Aloe vera* on diabetic induced group showing HDL-C level (n=6, values are mean ± S.D).

DISCUSSION

The currently available drug regimens for management of DM have certain drawbacks and therefore, there is a need to find safer and more effective anti-diabetic drugs [18]. DM of long duration is associated with several complications such as atherosclerosis, myocardial in fraction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood [19].

In the present study, alloxan (150 mg/kg b.w) was used for making diabetic model. Our results are similar to previous findings [20-22]. Alloxan selectively destroy pancreatic cell, after being taken up by the pancreatic cells via GLUT-2 glucose transporters, alloxan generates reactive oxygen species in a cyclic redox reaction with its reduction product, dialuric acid, autoxidation of which generates superoxide radicals, hydrogen peroxide and, in a final iron catalyzed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the β-cells [10].

In this study, we have demonstrated its antidiabetic properties, significantly lowering blood glucose in the alloxan-induced diabetes to values that are comparable to those of the non-diabetic control consistently for a period of 3 weeks. The glucose lowering effects were even more pronounced. This result was consistent with other co-workers [23-25].The antidiabetic properties of *Aloe vera* is comparable to those of several plants which have been demonstrated to possess hypoglycaemic and antidiabetic actions, for example, significant antidiabetic and antihyperlipidaemic effect was reported with neem seed (*Azadirachta indica*) on alloxan induced diabetes in rats [26],while the leaves extract was found to antagonize the glycogenolytic effects and increased peripheral utilization of glucose by epinephrine in alloxan and streptozocin induced diabetic and normal rats [27-28]. Similarly, chronic administration of crude aqueous extracts of *Momordica charantia* and *Swertia chirayita* also showed hypoglycemic effect in streptozocin treated rats and mice. Although *S. chirayata* was more effective [29]. Aqueous leaves extract of *Murraya koenigii* (curry leaf) as also been demonstrated to lower the lipid profile of alloxan induced diabetic rats [30], thereby reducing the risk of cardiovascular complications in diabetes as a result of its antioxidant properties. *Eruca sativa* seed oil also ameliorates the oxidative damage induced by alloxan diabetic rats as a result of its antioxidant properties [31].

It may be followed that the extract acts as an antioxidant blocking the formation of the reactive oxygen species; mechanism of which remains to be elucidated. Following treatment of the alloxan-induced diabetes, apart from its ameliorative effects on the blood glucose of the diabetic mice.

Hyperlipidemia is a known complication of DM and coexists with hyperglycemia and is characterized by increased levels of cholesterol, triglycerides and marked changes in lipoprotein fractions [32]. Control of hyperlipidemia is a prerequisite for the prevention of diabetic microangiopathy (retinopathy, nephropathy and neuropathy) and macroangiopathy (ischemic heart disease), cerebral vascular disease and arteriosclerosis obliterans in diabetes [33]. In the present study, elevation in serum lipid profile with concomitant decrease in HDL-C in alloxan-induced diabetic animals is in agreement with previous studies regarding alteration of these parameters under diabetic condition [34]. The biochemistry of the movement of lipids in the blood stream and the factors that increase lipid deposition in arteries is extremely complex. As far as cholesterol is concerned, the two lipoproteins most concerned with its transport are the high density lipoproteins (HDL) and the low density lipoproteins (LDL). LDL transports cholesterol to the cells where it is deposited even though it may not be required and is therefore associated with atherosclerosis. HDL, on the other hand, transports cholesterol to the liver where it can be removed from the body [35]. Normally, it is found that high cholesterol levels are associated with high LDL levels, but having a high HDL may compensate for this. In this respect, the markedly increased level of triglycerides and LDL-cholesterol in the serum of diabetic mice of the present work may be a consequence of either overproduction by the liver or defective removal from the circulation or both secondary to insulin deficiency [36]. Mechanisms by which HDL decreases in diabetes may be due to the impaired metabolism of triglycerides rich lipoprotein with decreased activity of lipoprotein lipase and

impaired transfer of materials to the HDL components, in addition to the high level of hepatic lipase among diabetics [37]. Thomas showed a strong relationship between high level of total cholesterol concentration in the blood and cardiovascular disorder [38]. Furthermore diabetics have an increased risk of coronary disorder [39-40]. However, administration of *Aloe vera* significantly reduced the lipid profile level and increased the HDL-C level. It was presumably mediated by a control of lipid metabolism. These results are similar to previous reports [41-42].

CONCLUSION

Results from this study have confirmed the hypoglycaemic and hypolipidemic efficacy of leaf extract of *Aloe vera* in mice. Further studies will be necessary for characterisation and structural elucidation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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