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Evaluation of Anti-hyperglycaemic Action of Different Fractions and Sub-fractions from Aqueous Extract of *Aloe vera* Linn. Leaf on Alloxan Induced Type 2 Diabetic Rats.

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ABSTRACT

Objective: To evaluate the fasting serum glucose (FSG) lowering potential of different fractions (C & D) and subfraction (D1 & D2) from aqueous extract of *Aloe vera* leaf on normal and alloxan induced type 2 diabetic rats. **Methods:** Two fractions (C & D) obtained by common chemical treatment of the aqueous extract of *Aloe vera* leaf and subfraction (D1 & D2) from fraction D were administered to the alloxan induced (150mg/kg i.p.) diabetic rats. The FSG lowering capacity, of different fractions and subfractions, was then evaluated in terms of percentage reduction in blood glucose level. **Results:** Oral administration of fractions C & D and subfraction D1 & D2 for 15 days led significant ($P < 0.05$) reduction to the elevated FSG level of alloxan induced diabetic rats. Percentage reduction in blood glucose level and comparison with standard drug glibenclamide suggest the superiority of fraction D and subfraction D1 in hypoglycaemic potential. **Conclusions:** The results suggest that fraction D and subfraction D1 from aqueous extracts of *Aloe vera* leaf possesses the maximum FSG lowering capacity and further investigation is required for determination of anti-diabetic principal(s) and exact mechanism of their hypoglycaemic action.

1. Introduction

Diabetes mellitus was recognized as early as 1500 B.C. by Egyptian physicians, who described it a disease associated with “The passage of much urine” [1]. It is one of the most dreadful and expensive disease facing the nations at present and the disease continues to grow vigorously in an alarming rate as an ‘epidemic’ [2]. In USA, more casualty has been reported by diabetes rather than any other disease including accidents. In China, diabetes is one of the three leading dreadful killers of middle age and elderly people. According to WHO in India there is fastest growing diabetic population and the country has been placed at forefront in the entire world. It is estimated that from 1995 to 2025, diabetic patients in India will increase by 195%.

Diabetes mellitus (DM) is a group of metabolic diseases

characterized by hyperglycaemia, hyper-triglyceridaemia and hyper-cholesterolaemia, resulting from defects in insulin secretion or action or both [3]. It is if uncontrolled results, consistently into high levels of blood glucose (>200mg/dl) leading to micro and macro vascular disease complications [4, 5], such as, blindness, lower extremity amputations, ultimate renal disease state, coronary heart disease and stroke through a diverse ways. Successful management of DM hinges on a multidisciplinary healthcare approach which includes a combination of diet, drug and or insulin therapy, exercise and behaviour modification to ensure long term compliance. However the consumption of synthetic medicines, for longer period, is either too expensive or associates much undesirable side effects which limits its popularity for treating chronic disease like DM. Thus in contrast the use of plant derived biomedicines is getting popularity day by day.

Aloe vera Linn., a popular houseplant, of family Liliaceae, has a long history as a multipurpose folk remedy. The genus *Aloe*, with nearly 420 species confined mainly to

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Africa, has over the years proved to be one of the most important sources of biologically active compounds [6]. It is an amazing mixture of more than 200 constituents, and commonly understood as store houses of many secondary metabolites, of medicinal importance, belonging to different classes of compounds. The common classes of compounds present in *Aloe vera* are, alkaloids, anthraquinones, pre-anthraquinones, anthrones, bianthraquinoids, chromones, flavonoids, coumarins and pyrones. Right from the inception of Ayurvedic, Chinese and other old medicinal systems to date now the leaf of *Aloe vera* have been consumed for the several potential clinical benefits. The major uses of *Aloe vera* are such as laxative [7], antibacterial [8], immune modulator [9], antioxidant [10] and anti-hyperglycaemic [11] etc. However despite of vast knowledge of variety of phyto-constituents and ethnomedical reports attributed to this plant, no detailed study has been carried out to describe its role in management of DM.

In light of this fact our previous study was performed to investigate the anti-diabetic extract from *Aloe vera* leaf which showed the aqueous extract was superior among other extracts in blood glucose lowering capacity [12]. In present work we extend the study to find out the fractions and subfractions from aqueous extract retaining the hypoglycaemic activity in alloxan induced type 2 diabetic rats.

Material & Methods:

Animals:

Six month old wistar albino rats of body weight 80–200 g of either sex were bred in the institutional animal house and used for the study. The animals were housed in standard polyacrylic cages and maintained under controlled room temperature ($22\pm 2^\circ\text{C}$), and relative humidity ($55\pm 5\%$) with 12 : 12 light and dark cycle. All the animals were provided with commercially available normal rat pellet diet (Phoster Biotech India, Ltd. Ambala) and water ad libitum. All experiments were performed in the morning according to the current guidelines for the care of laboratory animals and the ethical guidelines [13]. The guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) of the Govt. of India were followed and prior permission was granted from institutional animal ethics committee (Reg. No. 273/CPCSEA) for conducting the animal experimental studies.

Chemicals:

Alloxan was purchased from Loba Chemie Company, India. Glibenclamide was received as a gift sample from Ranbaxy Laboratories, Paonta Sahib, Himachal Pradesh. Tween 80, acetone, and ethanol were purchased from Rankem India Pvt Ltd. The GOD-POD kit and Dialysis Membrane was

purchased from Merck Laboratories.

Plant Procurement and Extraction:

Fresh leaves of *Aloe vera* were collected from Dr. Sushila Tiwari Herbal Garden, Muni Ki Reti, Tehri Garhwal, Uttarakhand, India. The species was authenticated from Botanical Survey of India, Northern Circle, Dehradun, India and voucher specimens (113504) was deposited in BSI, Dehradun, India for future reference. With appropriate storage condition the plant material was weighed and processed for extraction.

The fresh leaves of *Aloe vera* were washed and peeled to get the inside gel and pulp. 2.5kg of pulp was subjected to preparation of aqueous extract which was carried out by using soxhlet apparatus, with deionised water, for a week. The solvent was distilled off by evaporating at thin film evaporator and extract was dried to constant mass of 32g (1.28%).

Fractionation of Aqueous Extract:

20g concentrated aqueous extract was dissolved in sufficient water to make 200 ml volume of the solution. The solution was filtered with a cotton plug and acetone was added to the filtrate until precipitation stopped (approx 50ml). The solution was quickly filtered with vacuum filtration. To the filtrate 20 and 60% ethanol was added for further precipitation of polysaccharide. The polysaccharide fraction was separated out by vacuum filtration and supernatant was concentrated. This led the separation of 1.3g of polysaccharide fraction C (17%) and 16.6g of supernatant fraction D (83%).

Fraction D from aqueous *Aloe vera* leaf extract was showing maximum hypoglycaemic potential thence it was further subjected to fractionation with dialysis membrane (17.5 mm diameter). A 10 cm long piece of dialysis membrane was cut and left with excess of water for 72 hrs so that the membrane soaks water. 10g of the active fraction D was then dissolved in water to make 15 ml solution and the solution was then filled to the dialysis membrane. The membrane tied at both ends with the thread and left in the beaker filled with water. After 72 hrs when no further transport of any substance from dialysis membrane to water was found the fractions separated and concentrated. This led to the development of 6.8g sub-fraction D1 (68%) which was inside the dialysis membrane and 3.1g of sub-fraction D2 (31%) which was outside the dialysis membrane.

Both the sub-fractions were concentrated to fixed mass and evaluated for the anti-hyperglycemic potential on alloxan induced diabetic rats.

Pharmacological Study

Induction of diabetes:

Diabetes was induced by administration of alloxan monohydrates (150mg/kg i.p.) dissolved in normal saline to the overnight fasted rats [14]. After 72hrs of alloxan administration blood samples were drawn and glucose levels were determined by glucose oxidase peroxidase method to confirm diabetes induction. Rats having blood glucose level more than 150 mg/dl were considered as diabetic and taken for study.

Treatment Protocol:

The hypoglycaemic potential of fraction from aqueous extract was accomplished by dividing the rats into five groups containing five animals in each group. Group I, normal group of untreated rats; Group II, control group receiving tween 80 solution; Group III, standard group receiving 5mg/kg glibenclamide orally; Group IV, test group I receiving 75mg/kg of fraction C, and Group V, test group I receiving 75mg/kg of fraction D.

In a very similar fashion the treatment of diabetic rats were carried out with the subfraction D1 & D2. Group I, normal group of untreated rats; Group II, control group receiving tween 80 solution; Group III, standard group receiving 5mg/kg glibenclamide orally; Group IV, test group I receiving 75mg/kg of subfraction D1, and Group V, test group I receiving 75mg/kg of subfraction D2.

All the treatments i.e., fraction, subfractions and glibenclamide were administered orally as a suspension in 10% tween 80 solution for 15 days. On 1st, 5th, 10th and 15th day of study the animals were fastened overnight followed by withdrawn of blood by puncturing retro orbital plexus under mild ether anaesthesia. Serum was separated out by

centrifugation and fasting serum glucose level was analyzed through GOD–POD method [15].

Statistical analysis:

All values are presented as mean \pm SEM. Comparison between two groups was performed using student's t test. Multiple comparisons between different groups were performed using Analysis of Variance (ANOVA) followed by post hoc test for paired comparison. $P < 0.05$ was considered statistically significant. Statistical analysis was done with the help of Graph Pad Prism demo version 5 [16].

TLC and Qualitative Chemical Test:

Pre coated TLC plates (Al–Silica Gel 60 f 254) were dried in hot air oven for 1hr at 100–1100C. Approximately 5 μ g/ml solutions of subfraction D1 in water were prepared and spotting at the inert surface of stationary phase is applied with a small capillary. The diameter of spot was followed as per IP and the plates were then placed in a chamber containing small quantity of suitable solvent (known as developer) which serves as mobile phase. As the solvent reached to the top end of the plates, plates were removed from the chamber. The plates were then allowed to stand for 5min followed by spraying 10% alcoholic H₂SO₄ to visualize various compounds present in the sample mixture. The subfraction D1 was also subjected to qualitative chemical test for determination of nature of phyto–constituents present.

Results:

Table 1

Effect of fractions C and D of aqueous extracts of AV leaf extract on fasting serum glucose level of diabetic rats.

Group	Serum Glucose Level (mg/dl) in Days					
	Basal Value	1st	5th	10th	15th	
					Blood Glucose	% Reduction (15th day)
Normal Control	83.20 \pm 0.8	81.03 \pm 1.0	82.22 \pm 0.6	80.1 \pm 1.7	78.07 \pm 1.9	–
Diabetic Control	222.6 \pm 0.8*	223.5 \pm 1.5*	215.4 \pm 0.4*	214.4 \pm 1.3*	211.4 \pm 0.8*	5.03
Glibenclamide	265.9 \pm 1.2***	252.6 \pm 0.3***	202.9 \pm 1.2***	124.9 \pm 0.1***	90.9 \pm 1.2***	66.07
Fraction C	259.6 \pm 3.44***	253.0 \pm 3.6***	239.0 \pm 6.5***	213.9 \pm 2.14***	193.1 \pm 5.1***	25.61
Fraction D	249.6 \pm 5.8***	238.6 \pm 6.22***	203.2 \pm 7.24***	152.5 \pm 1.3***	113.5 \pm 5.3***	54.52

Value are in mean \pm SEM, No. of animals in each group N=6,, *Statistically significant different from normal group ($P < 0.05$), **Statistically significant different from Group II ($P < 0.05$), ***Statistically significant different from Group II ($P < 0.05$).

Table 2

Effect of fractions D1 and D2 of aqueous extract of AV leaf extract on fasting serum glucose level of diabetic rats.

Group	Serum Glucose Level (mg/dl) in Days					
	Basal Value	1st	5th	10th	15th	
					Blood Glucose	% Reduction (15th day)
Normal Control	89.08 \pm 0.3	87.31 \pm 1.6	87.28 \pm 1.6	86.7 \pm 1.5	85.07 \pm 1.6	–
Diabetic Control	232.1 \pm 0.2*	233.3 \pm 1.8*	225.4 \pm 0.9*	224.3 \pm 1.5*	221.8 \pm 0.4*	4.43
Glibenclamide	266.9 \pm 1.2***	250.6 \pm 0.3***	202.9 \pm 1.2***	124.9 \pm 0.1***	90.9 \pm 1.2***	65.94
Fraction D1	255.9 \pm 1.9***	242.6 \pm 0.8***	213.8 \pm 1.0***	143.9 \pm 1.2***	113.7 \pm 1.8***	55.56
Fraction D2	234.6 \pm 1.8***	228.6 \pm 1.0***	213.2 \pm 0.4***	178.5 \pm 0.3***	154.5 \pm 1.0***	34.14

Value are in mean \pm SEM, No. of animals in each group N=6,, *Statistically significant different from normal group ($p < 0.05$), **Statistically significant different from Group II ($P < 0.05$), ***Statistically significant different from Group II ($P < 0.05$).

The effect of fraction C & D and subfraction D1 & D2 in alloxan induced diabetic rats in 1st, 5th, 10th, and 15th day of treatment were summarized in table 1 & 2. A single intra-peritoneal dose of alloxan monohydrates (150mg/kg) led significant elevation in FSG level of diabetic rats as compared with normal control rats.

The results clearly showed that all the test groups containing fraction C & D and subfraction D1 & D2 showed significant ($P < 0.05$) reduction in FSG level. However fraction D caused maximum reduction of 54.52% as compared with the standard treatment (66.07%). The subfraction D1 also found to be most efficient on lowering FSG level (55.56%) when compared with the standard treatment (65.94%) (Figure 1 & 2).

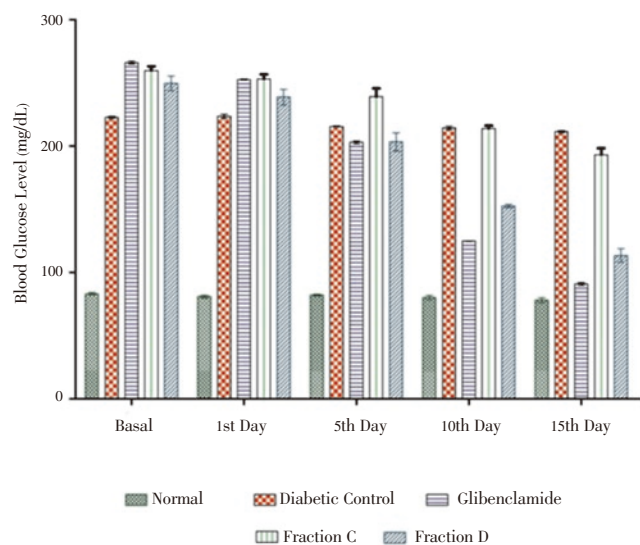


Figure 1: Effect of varlous fractions from AV teaves on FSG of diabetic rats

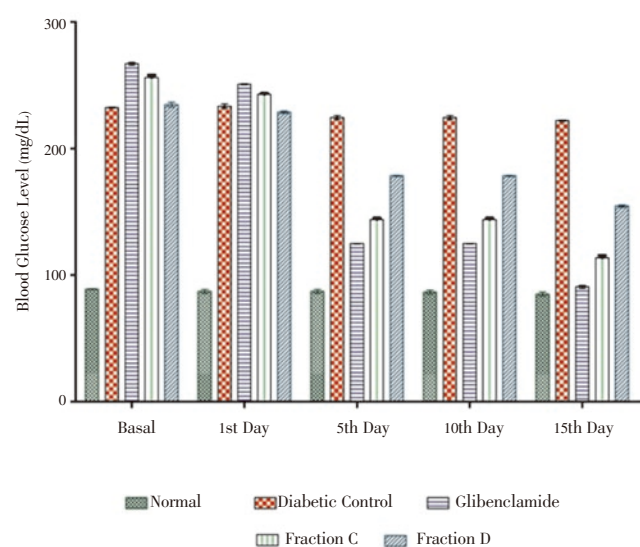


Figure 2: Effect of varlous subfractions from AV teaves on FSG of diabetic rats

Discussion:

Type 2 DM constitutes the majority of the diabetic cases. Unlike type I DM, a disease of insulin shortage, victims of type II DM usually have insulin in their bloodstream. In fact, insulin levels in type II diabetics are sometimes even higher than those in non-diabetic individuals. However, since the cells of a type II patient do not respond to insulin by binding it and utilizing blood glucose as normal cells do. Thus type II DM patients may have hyperglycemia in spite of high insulin levels. Oral hypoglycaemic drugs are used for the treatment of type 2 DM but because of some serious side effects associated with them these are not always recommended for prolong therapy [17,18].

Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of diabetes in many parts of the world. In present times, available evidence suggests a high prevalence of utilization of alternative medicine for the treatment of diabetes in some regions of the world [19–22].

The present study showed that fraction D and its subfraction D1 from aqueous extract of *Aloe vera* leaf ultimately retained the anti-diabetic potential. Thus D1 can be regarded as active anti-diabetic subfraction. The TLC study showed the presence of four compounds in the subfraction D1. Moreover, when subfraction D1 was subjected to qualitative chemical test it showed orange colour solution with dilute H₂SO₄, benzene, and dilutes ammonia confirming to the presence of anthraquinone glycosides present in the mixture sample [23]. The hypoglycaemic effect of subfraction D1 can be attributed to the anthraquinone glycosides present in it. Both glycosides [24] and anthraquinones have been reported to exhibit anti-hyperglycaemic action through diverse mode of action but most importantly the anti-hyperglycaemic effect of anthraquinones is expected through two major targets. Firstly, these are expected to modify the glucose transported 4 (GLUT4) expression in microtubes and secondly, through tyrosin phosphorylation of insulin receptors as influenced by tyrosin phosphatase 1B [25, 26].

From the results it could be concluded that the anti-diabetic potential of *Aloe vera* leaf is exhibited by the superfine fraction D1 which is a mixture of four components. The subfraction D1 also showed positive test for anthraquinone glycosides and the hypoglycaemic effect of *Aloe vera* leaf thus can be due to the presence of anthraquinone glycosides. However the anti-diabetic principal(s) and exact mechanism of their hypoglycaemic action requires further investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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