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DIABETIC REGULATION THROUGH BLOOD CONSTITUENTS' MODULATIONS ON TREATMENT WITH *ALOE VERA* IN ALLOXAN INDUCED DIABETIC RATS

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To investigate the protective use of ethanolic extract of *Aloe vera* (*AL*) on blood glucose levels, hematology in diabetic rats. The Protective effect of *AL* in experimental was evaluated in this study. Three months old male wistar rats were divided into 4 groups (n=6) namely: control, control+ *AL*, (300mg/Kg body weight) diabetic (Alloxan 40mg/kg body weight), diabetic + *AL* (300mg/kg body weight). Hematological and biochemical parameters were estimated after 21 days of experimental period. Body weights and blood glucose levels diabetic rats were significantly decreased in *AL* treated diabetic rats when compared to control rats. But body weights were significantly decreased with an elevation in blood glucose levels were noticed in diabetic rats over control rats. The blood parameters such as WBC, serum albumin, total proteins, cholesterol, urea and creatinine levels were significantly elevated in diabetic rats than control rats. However, the same blood constituents were reverted to the level of control on treatment with *AL* in diabetic rats. In contrast, the hemoglobin, RBC and albumin contents were lowered with a statistical significance in diabetic rats over control. On treatment with *AL* the rate of decrease was minimized in hemoglobin and elevated in RBC on treatment with *AL*. But the rates of depletion noticed in serum albumin levels of diabetic rats were showed much depletion in *AL* treated rats than control. The results of this study showed that *AL* extract given orally for 21 days attenuated the extensive beneficial changes of hematological, biochemical parameters were recorded in alloxan diabetic rats.

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Keywords: Antidiabetic, *Aloe vera*, hematology, blood glucose, alloxan, rats.

1. Introduction

Diabetes is a group of metabolic diseases with characteristic hyperglycemia associated with defects both in insulin secretion and insulin action [1]. Diabetes mellitus arises when insufficient insulin is produced, or when the available insulin does not function correctly. Without insulin, the amount of glucose in the bloodstream is abnormally high, causing unquenchable thirst and frequent urination, due to the body's inability to store or use glucose causes hunger and weight loss. Blood always has some sugar in it because the body needs sugar for energy to keep you going. But too much sugar in the blood is not good for our health [2]. Diabetes is associated with long-term (developing over many years) complications that affect on almost every major parts of the body. Diabetes causing stiffening and narrowing of very small blood vessels carrying oxygen to body cells and organs. And prolonged diabetes contributes to Blindness, Heart disease, Strokes, Kidney failure, Amputations, Nerve damage.

The most of biochemical pathways strictly associated with hyperglycaemia (non-enzymatic glycosylation, glucose auto-oxidation, and polyol pathways) and their abnormalities are

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high level leads to tissue damage and enzyme dysfunctions [3]. Liver is an important organ that plays a pivotal role in glycolysis, gluconeogenesis and glycogenolysis. A partial or total deficiency of insulin causes derangement in carbohydrate metabolism that causing impaired peripheral glucose utilization hepatic glucose production.

Plants have played a major role in the introduction of new therapeutic agents. Every ancient ethnic culture has its own treasure house of herbal medicines. At least [4], drugs from higher plants are being used throughout the world. Even now, almost 75-80% of world population depends on crude plant drug preparations to tackle their health problems[5].

Aloe Vere is also a remedy which has long been used in the Indian practice of Ayurvedic medicine. *Aloe vera* is belongs the Liliaceae family which are about 360 species [6]. It is commonly called aloe. *Aloe vera* is a succulent plant, mostly found in East and South Africa that has been used medicinally for centuries both internally and externally, to treat a wide variety of wounds including traumatic wounds, burns, lacerations, abrasions, punctures, sunburns, inflammation, and frost bite. It has been used to treat skin disorders like psoriasis, a rare skin disease known as lichen Planus, [7] and diabetic and pressure ulcers [8]. Internally, aloe may also be useful in the treatment of peptic ulcers [9]. Taken orally, *Aloe vera* gel helped reduce symptoms of patients with ulcerative colitis because of its anti-inflammatory effect [10].

2. Materials and methods

2.1 Experimental Animals

A total of 24 adults (3 months old) male Wister rats weighting 180±30g obtained from animal house of Bangalore were used for this study. They were housed spider each polycarbonate cage under standard laboratory conditions at a room temperature of 24±2degree centigrade humidity 45-64% with 12h Light/dark cycle. This study was carried out according to guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee at Sri Venkateswara university, Tirupati, India (No. 01/2011-2012/(i)/a/CPCSEA/IAEC/SVU/MB-SSR/Dt 20/06/2011).

The animals were fed on pellet diet (manufactured by Hindustan Lever Ltd., Bangalore, India) and water *ad libitum.*

2.2 Procurement of chemicals

All the chemicals used in the present study were Analar Grand (AR) and were obtained from following scientific companies sigma (st.couis, mo, USA) fisher (pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualiges (Mumbai, India)

2.3 Plant Material

Aloe vera (L) plants were collected from Tirumala hills. The taxonomic identification of *Aloe vera* (L) plant was confirmed by a senior Botanist, Mr. Madhava Chetty, Department of Botany, S.V. University and a voucher specimen (2011) was deposited in the herbarium.

2.4 Preparation of *Aloe vera* gel extract

Aloe vera extract was prepared from *Aloe vera* leaf gel according to the published procedure [11], with slight modifications. The fleshy solid gel in the center of the leaf was scratched with spoon, collected, homogenized and lyophilized. Then the lyophilized sample was extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator at 60°C. The residue was stored in dry sterilized small containers at 4°C until further use.

2.5 Experimental Design

The rats were divided in to 4 groups. Each group consists of 6 rats.

Group –I : Control rats.
Group- II : Control+*Aloe vera* (300mg/Kg BW of *Aloe vera* for 21 days).
Group –III : Diabetic rats (Alloxan 40mg/kg BW for a single dose).
Group –IV : Diabetic + *Aloe vera* (300mg/kg BW for 21 days).

2.6 Body Weight Changes

Body weights of all groups (4) of all rats were recorded before and after treatments. The body weights of all groups were recorded at an interval of one week till the completion of the experiential period (21days).

2.7 Estimation of Blood Glucose

Blood glucose was measured by using Accu Chek glucometer (Sensor Comfort) on 0 day, 10th day and 21st day of all 4 group rats.

2.8 Haematological and biochemical tests

The blood was collected into test tubes containing EDTA. RBC count was estimated by the haemocytometer method of [12], And White blood cells (WBC) count was estimated by the haemocytometer method of [13]. Hemoglobin was estimated by the method of [14]. Blood albumin, globulin, total proteins, creatinine, cholesterol, urea and creatinine were estimated using commercially available enzyme kits (span diagnosis, Gujarat, India).

2.9 Statistical analysis

All the grouped data were statistically evaluated with SPSS (Version 13.5; SPSS Inc, Chicago, IL, USA). One way analysis of variance (ANOVA) was carried out with Dunnet multiple comparison tests. *P*-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± S.D. for 6 animals in each group.

3. Results

3.1 Body Weights

Body weights of group-II the body weights were increased after the treatment with plant extract. In case of group-III the body weights were significantly decreased after induction with Alloxan. In the group-IV have showed decreased body weight levels. (Table-1.1).

Table: 1.1. Showing Body Weight levels in the control and experimental Animals

DAYS	Group I	Group II	Group III	Group IV
0 Day SD %	185 ± 10.0	183 ± 5.0 (-1.08)	195 ± 6.0 (+5.40)	200 ± 5.0 (+8.11)
10 Day SD %	220 ± 9.0	204 ± 4.3 (-7.27)	177 ± 5.4 (-19.54)	205 ± 5.72 (-6.81)
21 Day SD %	170 ± 5	171 ± 2 (+0.58)	174 ± 3 (+2.35)	179 ± 5 (+5.29)

Values are mean ± S.D. of 6 individual rats
Values in the parenthesis are % change from that of control
Values are significantly different from control at $P < 0.001$

3.2 Blood Glucose levels

Blood glucose levels of group-III blood glucose level were significantly increased after induction with Alloxan in group-IV where the rats were subjected to *Aloe vera* had showed decreased blood glucose level. The various blood glucose levels of alteration are as shown in (Table-1.2).

Table: 1.2. Showing Blood glucose levels in the control and experimental Animals

Days	Group I	Group II	Group III	Group IV
0 Weeks SD %	72.33 ± 9.93	70.83 ± 7.90 (-2.07)	466 ± 100.1 (+544.2)	394.67 ± 32.12 (445.6)
1st Week SD %	60.67 ± 7.76	80 ± 15.36 (+31.86)	377.17 ± 147.87 (+521.67)	155.83 ± 39.88 (+156.84)
2nd Week SD %	55.5 ± 17.02	59.5 ± 16.5 (+7.20)	263.67 ± 131.05 (+375.08)	72.33 ± 16.5 (+30.32)
3rd Week SD %	71.83 ± 18.85	62 ± 14.15 (-13.68)	293 ± 139.48 (+307.90)	82.5 ± 39.02 (+14.85)

Values are mean ± S.D. of 6 individual rats
Values in the parenthesis are % change from that of control
Values are significantly different from control at $P < 0.001$

3.3 Blood Parameters

A Significant decreased in the levels of total hemoglobin, during diabetes when compared with corresponding control groups. But WBC and RBC was increased in diabetes rats. Administration of *Aloe vera* extract tended to bring the values to near normal and the effect was more prone in the group of rats treated with *Aloe vera*.

A significant increase in serum proteins (5.42), albumin (2.84) and globulin (3.6) was recorded in *Aloe vera* diabetic group when compared to the untreated diabetic rats which showed significant decrease in scrum proteins, albumin and globulin levels 6.42, 3.12 and 4.8 respectively compared to the control group (Table-1.3).

Table: 1.3. Showing Blood Parameters levels in the control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
Hemoglobin gm/dl	12.8 ± 0.72	12.6 ± 1.46 (-1.5)	9.2 ± 1.86 (-28.12)	12.4 ± 0.98 (-3.12)
RBC millions/µl	4.5 ± 0.32	5.2 ± 0.29 (+15.55)	3.8 ± 0.37 (-15.55)	4.8 ± 0.43 (+6.66)
WBC cells/µl	5600 ± 348.23	6300 ± 296.34 (+12.5)	5800 ± 316.23 (+3.57)	6400 ± 361.24 (+14.28)
Albumin g/dl	3.52 ± 0.32	3.34 ± 0.26 (-5.11)	3.12 ± 0.48 (-11.36)	2.84 ± 0.36 (-19.31)

Parameters	Group I	Group II	Group III	Group IV
Globulin g/dl	3.4 ± 0.38	3.8 ± 0.42 (+11.76)	4.8 ± 0.97 (+41.17)	3.6 ± 0.82 (+5.88)
Total Proteins g/dl	5.68 ± 0.53	5.46 ± 0.69 (-3.87)	6.42 ± 0.92 (+13.03)	5.42 ± 0.34 (-4.58)
Creatinine mg/dl	2.16 ± 0.16	1.84 ± 0.34 (-14.81)	2.43 ± 0.42 (+12.5)	1.98 ± 0.68 (-8.33)
Urea mg/dl	28.96 ± 3.94	30.62 ± 8.29 (+5.73)	39.63 ± 6.46 (+36.84)	34.28 ± 5.98 (+18.37)
Cholesterol mg/dl	98.74 ± 10.92	109.43 ± 22.46 (+10.82)	142.38 ± 23.62 (+44.19)	106.68 ± 18.74 (+8.04)

Values are mean ± S.D. of 6 individual rats
Values in the parenthesis are % change from that of control
Values are significantly different from control at $P < 0.001$

Aloe vera treatment significantly decreased urea (34.28) and creatinine (2.43) respectively levels in diabetic rats compared to the untreated diabetic rats which shows significantly increased levels of urea (39.63) and creatinine (2.43) compared to control group. *Aloe vera* treatment significantly lowered serum cholesterols by 106.680 in the diabetic rats which were characterized by significantly raised levels of cholesterol (142.38) compared to the control rats.

4. Discussion

Body weight is determined by energy intake on one hand and energy experimental on the other. Imbalance between energy intake and expenditure results in a change in body weight. Alloxan induced diabetic rats shows decreased level of body weights. The decrease in body weight in diabetic rats clearly shows a loss (or) degradation of structural proteins. Weight loss which is one of the clinical features of diabetes mellitus may be due to the degeneration of the energy lost from the body due to frequent urination and oval conversion glycogen to glucose [15]. This shows that this plant extract in adipose the degeneration of the adiposite and muscle tissues which occurs during diabetic stress unordered to make up for the every lost from the body due to frequent urination and over conversion of glycogen urination and over conversion to glucose.

Glucose molecules are broken down with cells order to produce adenosine triphosphate (ATP) molecules, energy rich m les are delivered to cells by the circulating blood and therefore, to ensure a constant supply of glucose to cells, it essential that blood glucose levels be maintained at relatively constant levels. In the present study blood glucose levels were maintained at normal levels in control rats. Changes of the blood glucose levels in the group-II where normal rats were treated with plant extract are due to the alevonoid and triterpenoid compounds in then. The hypoglycemic effect of *Aloe vera* extract was induced only in hyperglycemic rats but not in normal glycemic animals [16]. Our observations stated that the hypoglycemic effect of *Aloe vera* was mediated through the simulation and release of insulin from beta-cells of the pancreas [17]. The hypoglycemic effect of *Aloe vera* extract was induced only in hyperglycemic rats but not in normal glycemic animals.

In the experimental diabetes enzymes of glucose and fatty acid metabolisim are markedly altered, hence blood glucose levels were increased [18]. Hyperglycemia is currently considered to primarily responsible for the free radicals, in particular the hydroxyl radical and low density lipoprotein oxidation [19]. The liver and other tissues are more resistant to reactive Oxygen

species comparison to pancreatic beta-cells and this resitance protects them against Alloxan toxicity [20, 21]. The formation of reactive Oxygen species is preceded by Alloxan reduction.

The results show a significant in plasma albumin, globulin, total protein and an increase in urea and creatinine levels in diabetic rats which in regrecennat with many earlier reports [22]. It is well known that in insulin deficiency (diabetes) decreased protein synthesis and increased protein degradation lead to release of amino acids which are directed for gluconeogenesis, Due to increased catabolism of proteins and amino acids.

Hepatic urea genesis and creatinine production are elevated in diabetic rats [23] lowered albumin and globulin in diabetic rats might be due to increased degradation and (or) decreased production and (or) increased urinary excretion of these substracts. *Aloe vera* supplementation appears to have rectified this abnormality in diabetic rats as evidenced by significantly elevated serum albumin levels in rats receiving *Aloe vera* observed that the risk of progression to over proteinuria can be reduction by improved glycemic control. In the present study also the glycemic control to the restored plasma albumin levels. The phyto chemicals of *Aloe vera* extract appear to have mitigated the metabolic abnormalities and restored the urea and creatinine levels. Though normal rats were also supplemented with *Aloe vera*, they did not show any difference in the levels of total proteins, albumin globulin, urea and creatinine indicating the selective potential therapeutic and anti diabetic effect of *Aloe vera* extract.

In response to blood glucose levels it is secreted by the beta-cell of the pancreas and extracts its effects by binding to cell surface receptors that are present on virtually all cell types and tissues [24]. The present study, normal rats treated with *Aloe vera* extract showed normal levels of insulin while diabetic rats has shown very low levels of insulin as a consequence of pancreatic beta-cell damage indicating low pancreatic beta-cell acting followed Alloxan.

5. Conclusion

In the present study, beneficial effects of *Aloe vera* and in Alloxan induced diabetic rats has been studied with selected blood serum parameters, body weight of rats. The decreased in body weight in diabetic rats clearly showed a loss or degradation of structural proteins. In the group-IV, body weights were gained near to control levels after treatment with *Aloe vera* plant extract. In *Aloe vera* and Alloxan groups respectively (Group-IV) blood glucose levels were decreased. This may be due to the antidiabetic compounds present in *Aloe vera* extract. However, the decreased levels were more significant in Group-IV. In Group-II the blood parameters such as Hemoglobin, RBC, WBC counts were similar as in controls where as highly decreased in Group-III (Diabetic rats) which suggest the anemic condition in the body, and increased count was observed in Group-IV (Diabetic + *Aloe vera*) . Group-IV shows increased levels when compared to (control rats) group-I. The triglyceride content which was observed high in diabetic condition was down regulated with *Aloe vera* treatment in the present investigation. This may be attributed to the increased metabolic utilization of triglyceride for the fuel use. Hence, triglyceride content was decreased in *Aloe vera* treated diabetic rats.

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