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Antidiabetic, Antihypercholestermic and Antioxidative Effect of Aloe Vera Gel Extract in Alloxan Induced Diabetic Rats

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Abstract: Aloe vera is used worldwide for several medical purposes as alternative medicine. The present study was aimed to evaluate the antidiabetic, antihyperlipidemic and antioxidative activity of aloe vera gel extract in diabetic and control rats. Forty male albino rats, weighing (95±5g) were divided into four groups; group 1: normal control, group 2: Diabetic control group (by Intraperitoneal injection of alloxan 100mg/Kg body weight), group 3: normal rats received aloe vera gel extract (0.5ml/day for 5 weeks) and group 4: diabetic rats received aloe vera gel extract (0.5ml/day for 5 weeks). Serum glucose, total cholesterol, triacylglycerols, Malondialdehyde (MDA), nitric oxide and total antioxidant capacity were estimated. Alloxan injection induced hyperglycemia, hyperlipidemia and oxidative stress. Oral administration of aloe vera gel extract resulted in a significant decrease in serum glucose, total cholesterol and triacylglycerols ($p < 0.05$) in treated diabetic group as compared with diabetic control group. In addition , treatment with aloe vera gel extract ameliorated the oxidative stress evidenced by a significant decrease in serum MDA level and a significant increase in serum nitric oxide and total antioxidant capacity ($p < 0.05$) in treated diabetic group as compared with diabetic control group. Trace element analysis of aloe vera gel extract showed that aloe vera gel extract contained appreciable amount of (Cr, Mn and Zn) which potentiate the antidiabetic activity of this plant. Moreover, aloe vera gel extract contained natural antioxidants (total phenols, total flavonoid, vitamins C and E) which are responsible for the antioxidative effect of this plant.

Key words: Antidiabetic; antihypercholestermic; antioxidative; aloe vera; rats.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, which results from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long- term damage, dysfunction and failure of various organs, especially the eyes kidney, nerves, hearts and blood vessels (American Diabetes Association, 2004). Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus in which diabetic patients experience various vascular complications such as atherosclerosis, coronary heart disease, diabetic nephropathy and neuropathy (Sheetz, 2002). There is increasing evidence indicating that enhanced production of free radicals may be an important contributing factor in the complications seen in diabetes. Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products, and enhanced glucose flux through the polyolpathway (Tiwari, 2002). Management of diabetes without any side effect is still a challenge to the medical system. This lead to increasing demand for natural products with antidiabetic activity and fewer side effects. Many herbs and plant products have been shown to have hypoglycemic action. Aloe vera is one of these antidiabetic plants (Grover, 2002). Aloe vera (Aloe barbadensis) known as “secret plant originated from ancient Egypt. The plant has been presented as a constituent of many phytochemicals vitamins, nutrients and anti-nutrients found in foods (Maenthalsong, 2007). Fresh aloe juice from the inner leaf parenchyma contains 96% water, polysaccharides (mucilage) consisting mainly of D-glucose and D-mannose, tannins, steroid, enzymes, plant hormones, amino acids, vitamins and minerals (Samulsson, 2004). Many of the health benefits associated with aloe vera have been attributed to the polysaccharides contained in the gel of the leaves. These biological activities include promotion of wound healing, antifungal activity, hypoglycemic or antidiabetic effects, anti inflammatory, anticancer, immunomodulatory and

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gastroprotective properties (Hamman, 2008). This study was carried out to investigate the antidiabetic, antihyperlipidemic and antioxidative activity of aloe vera gel extract in this plant.

MATERIAL AND METHODS

Animals and Experimental Design:

Adult male Wistar albino rats weighing (95g ± 5g) were purchased from Animal House of El-Salam-Farm, Giza, Egypt. Rats were housed individually with constant environment in controlled stainless steel cages, temperature (25°C ± 5°C) humidity(50% ± 10%), and light cycle were held constant 12/12 hr. The experimental period was 6 weeks on which food and water were provided ad libitum.

Animal's Diet:

Pellet diet (commercial diet) was used.

Composition of diet: protein 18 %, fat 4.9% and fiber 3.2%.

Preparation of Aloe Vera Gel Extract:

Fresh succulent leaves of aloe vera were collected, the inner gel component removed and the leafy exudate homogenized in an electric blender. This was subsequently lyophilized and stored at 4°C (Hamman, 2008).

Induction of Diabetes:

Diabetes was induced by a single intraperitoneal injection with alloxan (100 mg / Kg body weight) (Lenzen, 2008). Rats were fast overnight before injection with alloxan.

Experimental Design:

The animals were divided into 4 groups (each of 10 rats). All international and local rules and regulation for handling animals in experiments were followed. The experimental groups illustrated as follow:

Group 1: Healthy rats fed on pellet diet and served as normal controls.

Group 2: Diabetic rats fed on pellet diet.

Group 3: Healthy rats fed on pellet diet plus aloe vera gel extract (0.5 ml /day) served as positive control.

Group 4: Diabetic rats fed on pellet diet plus aloe vera gel extract (0.5 ml /day). At the end of the experiment, the animals were anesthetized with diethyl ether after 12 hours fasting and whole blood samples were taken from hepatic Portal vein. The blood samples left for 15 minutes at 37°C for serum separation, then centrifuged at 3000 rpm for 20 minutes, then sera were separated and kept in plastic vials at -20°C until analyses.

Biochemical Assays:

Serum glucose was determined by enzymatic colorimetric method according to the method described by Trinder (1969). Total cholesterol (TC) was determined in serum according to the enzymatic colorimetric method described by Richmond (1973). Triacylglycerols were determined in serum according to the enzymatic colorimetric method described by Fassati and Prencipe (1982). Malondialdehyde (MDA) was determined according to the colorimetric method described by Ohkawa *et al*. (1979). Nitric oxide was determined in serum according to the colorimetric method described by Montgomery and Dymok (1961). Total antioxidant capacity (TAC) was determined according to the colorimetric method described by Koracevic *et al*. (2001). MDA/TAC ratio was calculated as index of oxidative status.

Analysis of Aloe Vera Gel Extract:

Total phenols content were determined by Folin-Ciocalteu method described by Lafaka *et al*. (2007). Total flavonoids content were determined by Folin-Ciocalteu method according to method described by Meenakashi *et al*. (2009). Vitamin E was Colorimetry estimated by the method described by Mohamed *et al*. (2009).

Vitamin C was determined according to by the method described by Van and Emmeri (1936). Elements (Cr, Mn and Zn) were assayed by ICP spectrometer (ICAp 6000 series; Thermo scientific. Analysis of aloe vera gel extract was carried out in Faculty of Agriculture research Park-Faculty of Agriculture-Cairo University.

Statistical Analysis:

The data were presented as means ±SD. One way analysis of variance (ANOVA) followed by post hoc-least significant difference analysis (LSD) at ($p < 0.05$) was performed using the statistical package for social

science (SPSS) version 9 to compare all treated groups. Differences were considered to be significant when ($p< 0.05$).

Results:

The results in Table 1 and Fig .1 revealed that alloxan injection led to a significant increase in serum glucose level in untreated diabetic group compared with the corresponding control group ($P< 0.05$). Following oral administration of aloe vera gel extract , serum glucose level reverted back to near normal level. This increase in serum glucose level in diabetic group was accompanied by a significant increase in serum cholesterol and triacylglycerols in comparison with control group ($P< 0.05$). Oral administration of aloe vera gel extract caused a significant decrease in these elevated levels. Serum total cholesterol and triacylglycerols decreased by 31 % and 20.61 %, respectively as compared to untreated diabetic group.

Table 1: Serum glucose, total cholesterol and triacylglycerols (mg/dl) in different experimental groups.

Rat groups parameter	Group 1	Group 2	Group 3	Group 4
Glucose (mg/dL)	82.58 ± 9.8	a202.43 ± 7.31	b 83.45 ± 4.59	a,b,c 96.23±9.05
Total cholesterol (mg/dL)	70.76± 5.76	a 110.74 ± 8.44	b 68.06 ± 5.84	b,c 76.06 ± 5.19
Triacylglycerols (mg/dL)	71.93 ± 4.17	a 93.86 ± 2.99	b 72. 77± 7.65	b 74.50 ± 8.73

Significant difference ($P < 0.05$): compared to: (a): to group 1, (b): to group 2, (c): to group 3

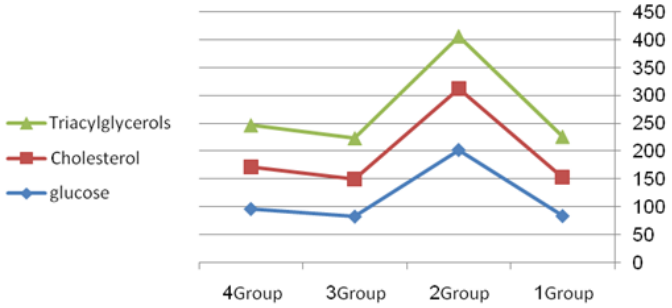

Fig. 1: Serum glucose, total cholesterol and triacylglycerols (mg/dl) in different experimental groups.

Induction of diabetes by alloxan injection caused a significant increase in MDA level. As shown in Table 2 and Fig. 2 serum MDA level was twice as high in untreated diabetic group than in control group (4.11 Vs 2.11 µ mol/L).Also, results reported that there is a significant increase in MDA/TAC ratio in untreated diabetic group as compared to normal control group. Due to aloe vera gel extract treatment these levels were brought close to that in the control group. The results illustrated in Fig. 3 demonstrated that there was a significant decrease in serum nitric oxide and total antioxidant capacity in untreated diabetic group relative to control group ($P< 0.05$).Oral administration of aloe vera gel extract increased serum nitric oxide level and total antioxidant capacity level to be near the normal levels.

Table 2: Serum MDA , nitric oxide (mmol/L) and total antioxidant capacity (M mol/L).

Rat groups parameter	Group 1	Group 2	Group 3	Group 4
MDA(mmol/L)	2.07 ± 0.31	a 4.11 ± 0.64	ab 1.46 ± 0.33	b,c 2.20±0.45
Nitric oxide(mmol/L)	49.74± 6.04	a 38.78 ± 5.82	b 54.78± 6.95	b 47.27 ± 4.59
Total antioxidant capacity(mmol/L)	2.18 ± 0.19	a 1.28 ± 0.35	b 2.54± 0.33	bc 2.61 ± 0.46
MDA /TAC ratio	0.81 ± 5.21	a 3.97 ± 1.05	b 0.86± 0.10	b 0.82 ± 0.28

Significant difference ($P < 0.05$): compared to: (a): to group 1, (b): to group 2, (c): to group 3

There were positive correlation between glucose and total cholesterol (r=0.924), triacylglycerols (r=0.820), while there was a negative correlation between MDA and total antioxidant capacity (r= -0.607) and nitric oxide (r=-0.275) as shown in Table 3.

Chemical analysis of some aloe vera gel components are summarized in Table 4. The results showed that aloe vera gel extract contained natural antioxidants such as total phenols (1.23 g/100g), Total flavonoids (0.71 g/100g), vitamins such as vitamin E (0.192 IU/100 g) and vitamin C (40mg/100ml).Trace element analysis showed that aloe vera gel extract contained Cr (0.306 mg/100g), Mn (0.594) and Zn (2.626 mg/g).

Table 3: Correlation coefficient between parameters.

Parameters	Correlation coefficient (r value)	Significance level
Glucose and TC	0.924	0.001
Glucose and TG	0.820	0.001
TC and TG	0.797	0.001
MDA and NO	- 0.275	0.05
MDA and TAC	- 0.607	0.05
TAC and NO	0.158	0.05

Table 4: chemical analysis for some aloe vera gel component.

Chemical composition	amount
Total phenols (g/100 g)	1.23
Total Flavonoid (g/100 g)	0.71
Vit. E (IU/100 g)	0.192
Vit. C (mg/100 ml)	40
Trace elements	(mg/100 g)
Cr	0.306
Mn	0.594
Zn	2.626

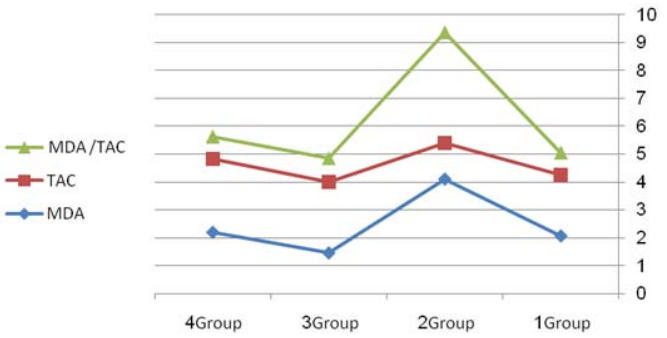

Fig. 2: Serum MDA, and total antioxidant capacity (M mol/L) and MDA/TAC.

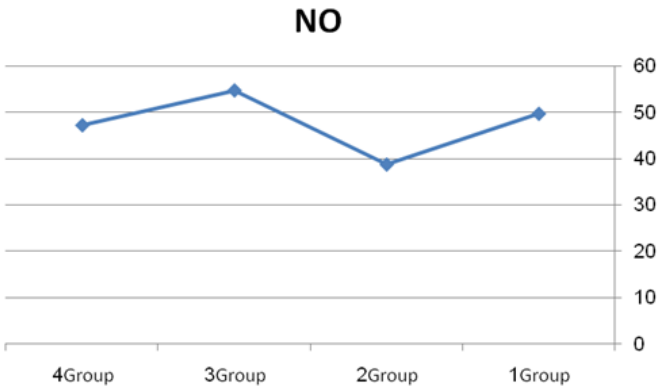

Fig. 3: Serum nitric oxide (µ mol/L).

Discussions:

Alloxan injection in rats caused a significant increase in serum glucose level in comparison with control rats. Oral administration of aloe vera gel extract reversed glucose level near normal. On the other hand when aloe vera gels extract was administrated to normal rats no significant drop in serum glucose level occurred. These findings suggested that hypoglycemic effect of aloe vera gel extract was evident only in diabetic rats but not in normal rats.

The results of this study are in accordance with the results of Gwarzo *et al*. (2010) who reported that intraperitoneal injection of alloxan induced diabetes.

The hypoglycemic action of the aloe vera gel extract was established by this study as has been demonstrated by other workers Akinmoladun and Akinloye (2004), who reported that a significant decrease in fasting blood glucose in alloxan-treated albino rats after oral administration of aloe vera for 30 days.

The results of Rajasekaran *et al*. (2004) and Noor *et al*. (2008) indicated that aloe vera gel extract was effective in lowering hyperglycemia in streptozotocin –induced rats.

The previous study of Abuelgasim *et al*. (2008) confirmed that antidiabetic action of aloe vera gel extract was induced only in hyperglycemic rats but not in normoglycemic rats which go hand in hand with results of the current study.

The results of Akinmoladun and Akinloye (2007) reported that aloe Vera extract prevent hyperglycemia in rabbits treated with alloxan. Also, Mohamed *et al*. (2009) confirmed that treatment with aloe vera juice filtrate resulted in a significant improvement in serum glucose in diabetic rats as compared to non diabetic control.

Diabetes is associated with hyperlipidemia. The results of this study showed significant increase in serum total cholesterol and triacylglycerols in untreated diabetic group .Treatment with aloe vera gel extract showed a hypolipidemic effect in diabetic group when compared with untreated diabetic group. The results of Rajasekaran (2006) supported the results of this study which confirmed that oral administration of aloe vera gel extract for 21 days resulted in a significant reduction in plasma cholesterol and triacylglycerols. Also, Kim *et al.* (2009) confirmed that administration of processed aloe vera gel for 8 weeks caused a significant decrease in serum triacylglycerols.

Oxidative stress plays an important role in the pathogenesis and the complications of diabetes. Hyperglycemia results in overproduction of oxygen free radicals, which contributes to the progression of diabetes. The development of complications during diabetes is also associated with oxidative stress. Alloxan act as a generator of reactive oxygen –species which are thought to be the major cause of B-cells dysfunction in diabetic animals induced by alloxan. Alloxan administration produced a marked oxidative impact as evidenced by the significant increase in serum lipid peroxidation product (MDA).These results are in harmony with the those reported by El-Missiry *et al*. (2004) and Ahmed (2009) which demonstrated that alloxan administration significantly increased lipid peroxidation product (MDA).

The present results indicated that induction of diabetes by alloxan produced a significant decrease in total antioxidant capacity. The present study confirmed the previous studies of Jackson *et al.* (2007) who found that the level of antioxidant capacity decreased in alloxan induced diabetic rats compared to normal control. They explained the decreased activities of total antioxidant capacity during diabetes may due to the production of ROS such as superoxide (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH).

The results showed that there was a significant decrease in serum nitric oxide in untreated diabetic rats when compared with normal control. The results of the current study is in the same line with that reported by Rajasekaran *et al.* (2005) who showed that induction of diabetes mellitus by alloxan in animals was associated with marked decrease in plasma nitric oxide.

The findings of this study revealed that oral administration of aloe vera gel extract have antioxidative effect which is manifested by the significant decrease in serum MDA levels with a significant increase in both serum nitric oxide and total antioxidant capacity.

These results are in accordance with those reported by Nwnajo *et al*. (2006) who confirmed that oral administration of aloe vera gel extract significantly decreased lipid peroxidation in tissues of diabetic rats. They explained the antioxidative effect of aloe vera gel in diabetic rats is due to reduction of blood glucose level, which prevents excessive formation of free radicals through various biochemical pathways. Also**,** El-sherbiny and Abdel- Aziz (2008) reported that treatment with aloe vera extract increased antioxidant activity as shown by significant decrease in lipid peroxidation product expressed as (MDA) in diabetic rats, they confirmed that male diabetic rats which fed 1% (per weight basis) freeze-dried aloe vera had a superior antioxidative action.

The results of Rajasekaran *et al.* (2006) demonstrated that total antioxidant capacity was significantly higher in aloe vera treated diabetic rats as compared to diabetic rats.

Trace element analysis showed that aloe vera gel extract contained appreciable amounts of Cr, Zn and Mn which may be responsible for potentiating insulin action. These trace elements present in aloe vera gel extract may play an important role in the control and management of diabetes since diabetes associated with marked alteration in the concentration of these trace elements. These elements are known as hypoglycemic elements because they have a very important role in glucose metabolism. Chromium facilitates insulin binding and subsequent uptake of glucose into the cell. Supplemental chromium has been shown to decrease fasting glucose levels, improve blood glucose tolerance, lower insulin levels and decrease total cholesterol and triacylglycerols Offenbacher and Pisunyer (1980).

The present study also demonstrated that aloe vera gel extract contained naturally occurring antioxidant components, including total phenols, flavonoids, vitamin C and vitamin E. These antioxidant components directly scavenge reactive oxidants, they are hypothesized to constitute a vital endogenous defense against

oxidative cell and tissue injury. Therefore, the natural antioxidants contained in a plant may contribute to the antioxidant activity and thus towards the total or partial alleviation of some clinical disorders in diabetes.

The results of Nwaguipe *et al.* (2010) showed that total polyphenols in aloe vera gel extract was (45.1±0.94 mg /100 g) and total flavonoids were (7.66±0.26 mg /100 g). Also, Botes *et al.* (2008) reported that aloe vera extract contained naturally occurring antioxidant components, including total phenols, flavonoids, ascorbic acid and α-tocopherol.

The study of confirmed that aloe vera gel contain Cr, Zn and Mn. Moreover, Josep (2010) and Moghaddasi and Verm (2011) summarized the chemical composition of aloe vera and their properties. Aloe vera gel extract contain inorganic compounds as Zn, Cr and Mn and vitamins as vitamin E and vitamin C.

From these results, the hypoglycemic effect of aloe vera gel extract may be due to the presence of hypoglycemic trace elements as Cr, Zn and Mn which potentiate insulin action. Also, the glucose lowering effect could be explained by the antioxidant activity of aloe vera gel extract because it attenuated oxidative damage in the serum of alloxan induced diabetic rats.

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