Research Article

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Anti-diabetic effect of aloe vera juice and evaluation of thyroid function in female diabetic rats

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This work aims to study the effect of diabetes mellitus on thyroid function and the efficacy of Aloe vera juice filtrate in ameliorating the glucose level and oxidative stress in alloxan injected rats. Eighty female albino rats were equally divided into 4 groups; I) non-diabetic rats represent the normal control group; II) experimentally diabetic rats injected with alloxan; III) diabetic rats induced by alloxan, followed by oral administration with 0.25 ml of Aloe vera whole leaf juice filtrate/Kg b.wt. 5 days/week for 4 weeks; IV) non-diabetic rats received only Aloe vera juice filtrates. Serum glucose, insulin, total T3, total T4, T3:T4 ratio, and total antioxidant capacity were estimated. It was found that serum glucose was highly significantly increased and serum insulin, total T3, total T4, total antioxidant capacity levels were highly significantly decreased in diabetic rats compared to the control. Treatment with Aloe vera whole juice filtrate resulted in a significant improvement in serum glucose, insulin and total antioxidant capacity in diabetic rats compared to non-diabetic control. In group III and group IV, T3 and T4 were significantly decreased compared to non-diabetic control group. It can be concluded that diabetes mellitus has been found to be associated with thyroid function disorder. In addition to the fact that Aloe vera has a hypothyroid effect on induced diabetic rats.

Key words: Aloe vera, Antioxidant Capacity, Diabetic rats, Thyroid functions.

Diabetes mellitus is recognized as one of the leading causes of morbidity and mortality in the world and characterized by hyperglycemia. Hyperglycemia is the most common serious metabolic disorder leading to various complications (Rahimi *et al*., 2005). There are three main known forms of diabetes mellitus; type I, type II, and gestational diabetes, which have different causes and population distributions. While, ultimately, all forms are due to insufficient production of insulin by the beta cells of the pancreas (Rother, 2007). There are other causes of diabetes mellitus that do not fit into the previous types; attempts to classify them remain controversial. Some cases including the body's tissue receptors not responding to insulin, genetic mutations can lead to defects in beta cell function, abnormal insulin action may also been genetically determined in some cases. Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (Alberti and Zimmet, 1998).

Alloxan and streptozotocin are chemicals which selectively destroy pancreatic β-cells (Islas-Andrade *et al*., 2000). Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) with molecular formula $C_4H_2N_2O_4$ is a pale reddish colour unstable organic compound based on a pyrimidine. It is an oxidative product of uric acid. The action of alloxan in the pancreas is preceded by its rapid uptake by the β-cells (Boquist *et al*., 1983). Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity. Another aspect concerns the formation of reactive oxygen species (ROS). Oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related complications. It became clear that ameliorating oxidative stress using antioxidants might be an effective strategy for reducing diabetic complications (Giugliano *et al*., 1996).

Pancreatic β-cells express low levels of antioxidant enzymes and do not

up-regulate these enzymes upon exposure to high concentration of glucose. Thus, increased ROS production with low antioxidant defenses could result in ROS accumulation and oxidative stress in β-cells. Elevated ROS affects the function and survival of β-cells through direct oxidization of cellular macromolecules such as DNA and lipids, and activation of cellular stess-sensitive signaling pathways (Wu *et al*., 2004). The antioxidant defense system represents a complex network with interactions, synergy and specific tasks for a given antioxidant. The efficiency of this defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage (Polidori *et al*., 2001).

Management of diabetes without any side effects is still a challenge to the medical system. This leads 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 (Grover *et al*., 2002). *Aloe vera* (Liliaceae.) (synonym *A. barbadensis* MILLER), is one of these antidiabetic plants. *Aloe* plant, being a cactus plant, is between 99 and 99.5% water, with an average pH of 4.5. The remaining solid material contains over 75 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Miyuki *et al*., 2006).

Diabetes mellitus and thyroid disorders involve a dysfunction of the endocrine system. Studies have found that diabetes and thyroid disorders tend to co-occur in patients (Coiro *et al*., 1997). Since insulin and thyroid hormones involved in cellular metabolism and so excess or deficit of either of these hormones could result in the functional derangement of the other (Donckier, 2003).

Complementary and alternative medicine claims that dietary supplements and herbal remedies are safer than conventional drugs. Aloe is widely used in a complementary herbal medicine approach. There isn't enough research in this area to know how well *Aloe vera* works when taken as a liquid or capsules that are swallowed, or as an injection and how it may affect thyroid metabolism in humans (Palmer *et al*., 2003). This work aims to study the effect of diabetes mellitus on thyroid function and the efficacy of *Aloe vera* juice filtrate in ameliorating the glucose level and oxidative stress in alloxan injected rats.

MATERIALS AND METHODS

A total number of eighty female albino rats (*Rattus norvegicus*) weighing 180 to 200 grams were purchased from the National Research Center, Dokki, Cairo, Egypt, and allowed to acclimate to the laboratory conditions for 2 weeks prior to experimentation. The animals were freely fed on standard rodent pellets and tap water offered *ad-libitum*.

The rats were equally divided into 4 groups: Group I consists of non-diabetic rats represents the normal control group. Group II consists of rats intraperitoneally injected with alloxan, purchased from Egypt Company for Chemicals as a single dose of 175 mg/kg body weight. Rats were fast overnight before injection with alloxan. Blood samples were withdrawn 5 days post-injection and animals with serum glucose level > 200 mg/dl were considered diabetic. Group III experimentally diabetic rats induced by alloxan, as in group II, followed by oral administration with 0.25 ml of Aloe vera juice filtrate/Kg body weight 5 days/week for 4 weeks using stomach tube. Group IV consists of non-diabetic rats received only Aloe vera juice filtrates for 4 weeks. All international and local rules and regulation for handling animals in experiments were followed.

After alloxan injection, the rats were allowed to drink 5% glucose solution for 24 hours to overcome the drug induced hypoglycemia. Blood samples were withdrawn 5 days post-injection from sinus orbitus vein. Mature, healthy and fresh leaves of *Aloe vera* were prepared daily in the laboratory by washing with fresh water then, filtering the whole juice of *Aloe vera* leaf plant after skinning the leaf with care taken to avoid contamination of the gel from the outer layer. Then, the whole juice was collected into a sterilized cup and blended before usage.

At the end of the experiment, rats were lightly anesthetized using diethyl ether and fasting blood samples were withdrawn by heart puncture after sacrificing the rats. The blood samples were allowed to clot at 37°C in a water bath. Serum was separated and kept frozen at -20 °C until assayed.

Serum glucose was determined by enzymatic colorimeteric method of Trinder, (1969). Determination of glucoseurea was carried out according to the method of Free and Free (1979). Serum total antioxidant

Table 1: Serum glucose, insulin and total antioxidant capacity levels in normal control rats (Gr.I), diabetic rats (Gr. II), diabetic rats treated with *Aloe vera* (Gr. III) and rats administered *Aloe vera* (Gr. IV).

Parameter	Glucose	Insulin	Total antioxidant capacity
Group I: (Non-diabetic control)	103.22 ± 14.51	12.05 ±1.80	1.13 ± 0.29
Group II: (Diabetic)	274.56 ± 39.77	4.92 ± 0.74	0.61 ± 0.11
P_1	≤0.001	≤0.001	≤0.001
Group III: (Diabetic + Aloe treated)	113.15 ± 14.30	10.71 ± 2.21	0.91 ± 0.12
P_1	≤0.05	≤0.05	≤0.01
P_2	≤0.001	≤0.001	≤0.001
Group IV: (Non-diabetic + Aloe treated)	106.19 ± 8.37	13.31 ± 1.74	1.37 ± 0.26
P_1	>0.05	≤0.05	≤0.01

P1: Compared to control group (Group I), P2: Compared to diabetic group (Group II).
No. of rats in each group = 20 rats. Presented values are mean ± S.D.

capacity was determined by colorimetric method described by Koracevic, (2001). Serum insulin, T_3 and T_4 levels were estimated by solid-phase radioimmunoassay technique (RIA) according to the method of Burtis and Ashwood, (1994), Hollander and Shenkman, (1974) and Wenzel, (1981) respectively.

Data were statistically analyzed using Microsoft Office Excel 2003 software for windows. Student's t-test was used to evaluate the difference between the means of two sets of data. P values < 0.05 were considered to indicate statistical significance.

RESULTS

Serum glucose level in non-diabetic control group (Gr. I) was found to be within normal level recorded 103.22 ± 14.51 mg/dl. In diabetic group (Gr. II) serum glucose level was very highly significant increased compared to that of control group (274.56 ± 39.77 mg/dl, p < 0.001, table 1), whereas, in diabetic + *Aloe vera* group (Gr. III) it was significantly increased (113.15 ± 14.30 mg/dl, p < 0.05, table 1) as compared to non-diacetic control group. (is this increase from 103.2 to 113.15 is realy significant please check it). Oral administration with 0.25 ml of *Aloe vera* juice filtrate/Kg b.wt. resulted in a very highly significant reduction in serum glucose level (106.19 ± 8.37 mg/dl) of diabetic rats when compared to that of diabetic group (p < 0.001, table 1). Due to *Aloe vera* treatment in diabetic rats, the level was brought close to that in the non-diabetic control group.

Serum insulin level in non-diabetic control group was 12.15 ± 1.80 µIU/ml as shown in table (1). The obtained result in diabetic group (II) revealed a very highly significant decrease (P < 0.001) in serum insulin (4.92 ± 0.74 µIU/ml) compared to the non-diabetic control (table 1).

Serum insulin level of group III was almost around the non-diabetic control level after *Aloe vera* treatment (10.71 ± 2.21 µIU/ml, P < 0.05). In comparison with diabetic group, there was a very highly significant increase (P < 0.001) in serum insulin level in Gr.III as observed in table (1). Statistical analysis of Gr. IV revealed a significant increase in serum insulin recording 13.30 ± 1.74 µIU/ml compared to the non-diabetic control (P < 0.05, table 1).it seems not to be significant please check it Serum total antioxidant capacity in diabetic group was highly significantly decreased when compared with non-diabetic control (p < 0.001, table 1). In diabetic rats with *Aloe vera* treatment (Gr. III), serum total antioxidant capacity was also lower (0.90 ± 0.12 mM/L) compared to the non-diabetic control. However, serum total antioxidant capacity was significantly higher in *Aloe vera* treated diabetic rats as compared to diabetic rats (p < 0.001, table 1).

In non-diabetic control group, serum triiodothyronine level was found to be within normal reference level (102.20 ± 14.03 ng/dl, table 2). Serum T_3 in diabetic group (56.10 ± 5.12 ng/dl) showed a very highly significant decrease (P < 0.001) compared to non-diabetic control group. The obtained results for group III also demonstrated a very highly significant decrease (56.43 ± 8.57 ng/dl, p < 0.001) in serum T_3 level compared to non-diabetic control group. In other words, no statistically significant alteration was recorded for serum T_3 between group II and III (table 2). A very highly significant decrease (58.948 ± 9.578 ng/dl, P < 0.001) in serum T_3 level was also observed in group IV compared to that of non-diabetic control.

Serum thyroxine level was 9.03 ± 1.88 µg/dl in non-diabetic control group (table 2). Regarding Gr. II, serum T_4 level (9.03 ± 1.88 µg/dl) showed a very highly significant decline (p < 0.001) compared to

Table 2: Serum triiodothyronine (T_3), thyroxine (T_4) levels and T_3: T_4 ratio in normal control rats (Gr.I), diabetic rats (Gr. II), diabetic rats treated with *Aloe vera* (Gr. III) and rats administered *Aloe vera* (Gr. IV).

Parameter	Triodothyronine level (T_3)	Thyroxine level (T_4)	T_3 : T_4 ratio
Group I: (Non-diabetic)	102.20 ± 14.03	9.03 ± 1.88	11.55 ±1.40
Group II: (Diabetic)	56.10 ± 5.12	4.41 ± 0.86	13.02 ±1.64
P_1	≤0.001	≤0.001	≤0.01
Group III: (Diabetic + Aloe treated)	56.43 ± 8.57	3.83 ± 0.85	15.21 ±3.18
P_1	≤0.001	≤0.001	≤0.001
P_2	>0.05	>0.05	≤0.01
Group IV: (Non-diabetic + Aloe treated)	58.95 ± 9.58	4.31 ± 0.86	13.99 ±2.41
P_1	≤0.001	≤0.001	≤0.001

P1: Compared to control group (Gr.I) , P2 : Compared to diabetic group (Gr.II).
No. of rats in each group = 20 rats. Presented values are mean ± S.D.

non-diabetic control. The hormonal evaluation in diabetic + *Aloe vera* group (Gr. III) showed a very highly significant decrease in T_4 level (3.83 ± 0.85 µg/dl) in comparison with the non-diabetic control. However, its level was insignificantly changed compared to group II (table 2). A very highly significant decrease ($p < 0.001$) in serum T_4 level was observed in group IV (4.31 ± 0.85 µg/dl) compared with that in non-diabetic control.

In non-diabetic control group, T_3:T_4 ratio was 11.55 ± 1.40 (table 2). Diabetic group (Gr. II), T_3:T_4 ratio was highly significantly increased (13.02 ± 1.64, $p < 0.01$) compared to non-diabetic control. A highly significant increase (15.21 ± 3.18 $p < 0.01$) in T_3: T_4 ratio was observed in Gr. III as compared to the non-diabetic control. A highly significant ($p < 0.01$) increase in T_3: T_4 ratio in Gr. III was observed when compared with the diabetic group. In Gr. IV T_3:T_4 ratio was very highly significantly increased (13.99 ± 2.41) compared to Gr.I ($p < 0.001$).

DISCUSSION

The present finding concerning elevated serum glucose and decreased serum insulin levels is in agreement with Ramalingam and Leelavinothan, (2005) and Subbiah *et al*. (2006) who indicated that alloxan injection caused diabetes by rapid depletion of β-cells, which leads to a reduction of insulin release and elevation in blood glucose. Rapid uptake by insulin-secreting cells is the most factor determining alloxan diabetogenicity. The previous study of Szkudelski, (2001) concluded that the formation of reactive oxygen species (ROS) including H_2O_2 is preceded by alloxan reduction, which occurs in the presence of different reducing agents in the β-cells of the pancreas.

The ameliorative effect of *Aloe vera* whole juice filtrate in decreasing serum glucose level and a marked increasing of serum insulin supports the finding of Subbiah *et al*. (2005) who found that the diabetic rats received *Aloe vera* gel extract exhibited a marked elevation in serum insulin level and a significant reduction in blood glucose. The results also are in harmony with the findings of Pérez *et al*. (2007) and Loots *et al*. (2007) who reported that oral administration of ethanolic extract from *Aloe vera* leaf gel to induced diabetic animals significantly restored the glucose level. They suggested that the anti-hyperglycaemic activity of *Aloe vera* could be due to an insulinogenic activity of the *Aloe vera* whole juice filtrate ingredients including phytosterols, glucomannan, a hydrosoluble fiber, aloin, and aloe-emodin.

In our previous study it was demonstrated that diabetic rats were characterized by hyperlipidemia and oxidative impact as evidenced by the significant increase in lipid peroxidation (serum TBARs level) give reference of your previouse study here. This might reflect an inhibitory action of alloxan on both enzymatic and non-enzymatic antioxidant system (Pari and Saravanan, 2007). In diabetes, the increase in oxygen free radicals could be due to an increase in levels of blood glucose, which upon auto-oxidation generates free radicals. Insulin secretion is also closely associated with lipoxygenase-derived peroxides. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. Its products (lipid radical and lipid peroxide) are harmful to the cells in the body and associated with atherosclerosis. In present study, alloxan administration produced a significant decrease in total antioxidant capacity, indicating an increasing in production of reactive oxygen species. The antioxidant capacity is a measure of the total protective

antioxidant mechanisms in the body, both for preventing the production of free radicals and for repairing oxidative damage (Koracevic *et al*., 2001). Alloxan-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage. Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cell and protect pancreatic islets against the cytotoxic effects of alloxan (Coskun *et al*., 2005).

Our results of total antioxidant capacity is in agreement with that of Jackson *et al*. (2007) who established that the level of total antioxidant capacity significantly decreased in alloxan-induced diabetic Sprague-Dawley rats. They found that the decreased activities of total antioxidant capacity during diabetes mellitus may be due to the production of ROS such as superoxide (O_2•), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•). The present result is also consistent with Takayuki *et al*. (2008) who found that antioxidant capacity was decreased significantly in diabetic rats compared to normal control. It was suggested that oxidative stress increased at a very early stage of diabetes and oxidative stress, is determined by the overall antioxidant capacity.

The present work demonstrated that administration of *Aloe vera* whole juice filtrate/Kg b.wt. to diabetic rats led to a significant increase in total antioxidant capacity. The present finding is in harmony with those reported by Lim *et al*. (2003) and El-Sherbiny and Abdel-Aziz, (2008) who reported that the male diabetic rats which fed 1% (per weight basis) freeze-dried aloe filet had superior anti-oxidative action against lipid peroxidation, as indicated by reduced levels of hepatic phosphatidylcholine hydroperoxide.

Diabetes mellitus and thyroid diseases are the two common endocriniopathies seen in the adult population. There is inter-dependence between insulin and thyroid hormones for normal cellular metabolism so that diabetes mellitus and thyroid diseases can mutually influence the other disease process (Sathish and Mohan, 2003). Results showed that diabetes mellitus was found to be associated with abnormalities of thyroid function tests; including a significant decrease in serum total triiodothyronine (T_3) level and serum total thyroxine (T_4) level. T_3:T_4 ratio was highly significantly increased in diabetic group compared to the normal control. The current results confirm previous observation of Ali *et al*. (2004) who found a decrease in serum T_3 and T_4 in diabetic rats compared to control group. Donckier, (2003) reported that in euthyroid individuals with diabetes mellitus, the serum T_3 levels, T_4 levels, basal TSH and TRH levels may be strongly influenced by the glycemic status. Low serum T_3 is due to reduced peripheral conversion of thyroxine (T_4) to tri-iodothyronine (T_3) via $5^{P`}$-monodeiodination reaction.

The obtained results of T_3 and T_4 in diabetic group are in harmony with those of Jatwa and Kar (2006) and Hamendra and Anand, (2007) who found a significant decrease in serum levels of T_3 and T_4 in experimental animals. They reported that diabetes mellitus has a strong inhibitory effect on the deiodination of T_4 as well as the stepwise deiodination of the other partially iodinated thyronines. The inhibition of iodothyronine deiodination in the periphery that leads to reduced T_3 production from T_4 appears to be by far the dominant influence of diabetes mellitus. In addition, diabetes may be associated with a primary impairment of thyroid hormone secretion.

The present data in Gr. IV revealed that oral administration of *Aloe vera* caused a significant decrease in serum T_4 and T_3 level when compared to normal control. These findings are in agreement with those of Kar *et al.* (2002) who found that in Swiss albino male mice administered plant extract of *Aloe vera,* both serum levels of T_3 and T_4 decreased significantly. They mentioned that *Aloe vera* extract decreased both T_3 and T_4 concentrations by about 25 and 13%, respectively through an unknown effect. The present findings are consistent with El-Sherbiny and Abdel-Aziz, (2008) who stated that there are significant decreases in serum levels of T_3 and T_4 in rats administered of *Aloe vera* juice filtrate compared to control rats. Similarly, a 56-year-old woman case study by Pigatto and Guzzi (2005) displayed a decreased level of T_3 and T_4 after treatment with *Aloe vera* plant juice at doses of 10 mL/day for 11 months.

On the basis of the present findings, it can be concluded that diabetes mellitus has been found to be associated with thyroid function disorder; including a decrease in serum T_3 and T_4 levels. *Aloe vera* can ameliorate the hyperglycemia, whereas it has a hypothyroid effect on induced diabetic rats. More studies are needed to know how

well *Aloe vera* may affect the levels of thyroid hormones in humans.

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