World Journal of Zoology 6 (1): 07-11, 2011
ISSN 1817-3098

Effect of Apple Vinegar on Physiological State of Pancreas in Normal and Alloxan Induced Diabetic Rats

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Abstract: Apple vinegar, a folklore anti-diabetes fern, was evaluated for its hypoglycaemic and anti-diabetic properties using rats. The blood glucose lowering activity of the apple vinegar was studied in normal and alloxan-induced diabetic rats. Blood samples were collected from the tailvein of rats and serum glucose measured on the 1st, 7th, 14th days of the experiment. The apple vinegar produced dose-dependent reduction in blood glucose of both normal and diabetic rats and was comparable with that of the standard drug, Glibenclamide. A significant reduction in blood glucose of diabetic animal's 8.3±0.75 mmol/L in case of administration of Apple Vinegar in comparison with diabetic control 24.6±8.45 mmol/L, while Glibenclamide drug produced a reduction in blood glucose 12.27 ± 4.77 mmol/L. Results indicated a prolonged action in reduction of blood glucose by apple vinegar and the mode of action of the active compounds of apple vinegar is probably mediated through enhance secretion of insulin from the β-cells of Langerhans or through extrapancreatic mechanism.In conclusion, the present study clearly indicated a significant antidiabetic activity with the apple vinegar and supports the traditional usage of the apple vinegar for activation of pancreatic β cells and control of diabetes.

Key words: Diabetes Mellitus · Glibenclamide · Hypoglycaemic agent

INTRODUCTION

Diabetes mellitus is one of the chronic diseases affecting about 1% of the Western countries and 5-10% of the world population [1]. Diabetes characterized by decreased insulin sensitivity leading to insulin resistance in its target tissues [2, 3]. On the other hand, impaired glucose-induced insulin secretion with a decrease in pancreatic β cell mass will eventually lead to chronic hyperglycaemia [4,5]. In spite of the hypoglycemic agents, diabetes and the related complications continue to be a major medical problem [6].

Since time immemorial, patients with non-insulin dependent *Diabetes mellitus* have been treated orally by folklore with a variety of plant extracts. In the indigenous Jordan system of medicine, a mention was made on good number of plants and compounds for the cure of diabetes and some of them have been experimentally evaluated and the active principles were isolated [1]. However, search for new anti diabetic drugs continues.

Apple vinegar products are advertised in the popular press and over the internet internet for treatment of a variety of conditions, including: aging, weight loss, hemorrhoids, high blood pressure, arthritis, sore throat and diabetes [7]. Apple vinegar is a powerful detoxifying and purifying agent, it breaks down fatty, mucous and phlegm deposits within the body. By breaking down these substances it improves the health and function of the vital organs of the body, such as the kidneys, bladder and liver, by preventing excessively alkaline urine. It also oxidizes and thins the blood, which is important in preventing high blood pressure [8]. Antiglycemic effect of apple vinegar was seen after consumption of a starch load co administered with a 2% acetic acid solution [9, 22].

Simple, inexpensive diet strategies to help manage blood glucose are greatly needed to delay the progression of diabetes. Accumulating evidence indicates that a single dose of Apple vinegar may attenuate postprandial glycemia (PPG). Since PPG is a strong predictor of hemoglobin A1C, particularly in well controlled diabetic patients [10]. Simple but effective ways to lower blood glucose levels, applicable to a wide variety of dietary habits, are strongly needed. A longitudinal trial has not been conducted to determine whether regular Apple Vinegar ingestion has therapeutic value for individuals with diabetes.

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In the present study, the apple vinegar has been evaluated for hypoglycemic activity in normal and alloxan diabetic rats.

MATERIALS AND METHODS

Samples and Chemicals: Apple (*Malus domestica*) vinegar was obtained from the supermarket in Irbid City, Jordan. Acetic acid contents of the vinegar was 4.5% and solid contents was 13.3%.

Glibenclamide was provided by Diamond Pharmaceutical company, Amman, Jordan, while alloxan (Sigma Chemical Company, USA). All other reagents used were of analytical grade.

Animal Experiments: Rats of original Wistar strain bred in the Central Animal House, Department of Biological sciences, College of Science, Yarmouk University were used in this study. Experiments were carried out in male rats weighing between 180 and 220 g. Rats provided with a standard diet and water *ad libitum*. All they kept in cages with Wide Square mesh at the bottom and maintained in a well-ventilated animal house with 12 h light and dark cycle. They were fasted for 18 h prior to the experiment, allowing access to water only and were deprived of both food and water during the 24 h monitoring period of the experiment after the treatment either with the drug or distilled water (control) to minimize the changes in plasma volume.

Induction of Experimental Diabetes: Diabetes was induced in the rats by a single intraperitoneal injection of alloxan (150 mg /kg body weight). Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15-20 ml) intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia [11]. Diabetes was confirmed by the use of an Ames One Touch Glucometer (LifeScan; Johnson and Johnson, New Brunswick, NJ). After 5 days when the condition of diabetes was stabilized, rats with blood glucose range of 200-300 mg/dl were selected for the study.

Experimental Procedures: Serum glucose was spectrophotometrically estimated using a commercial assay kit (Diamond, Jordan Ltd.). Blood glucose values were measured on the 1st, 7th, 14th days of the experiment. Rats were divided into five experimental groups of five rats each:

Group 1: Received normal saline and served as control.

Group 2: Received Apple vinegar orally through intragastric intubation at doses of 2 ml/kg body weight and served as control.

Group 3: Diabetic rats received 10 ml/kg tap water and served as diabetic control.

Group 4: Diabetic rats received orally apple vinegar 2 ml/kg and served as treated group.

Group 5: Diabetic rats were given glibenclamide 600 µg/kg body weight [12] in aqueous solution daily using an intragastric tube for 14 days.

Collection of Blood: Blood samples (approx. 0.3 ml) were collected from tail veins of each rat of a group on 0, 7, 14 days after oral administration of the drug. The samples were collected into plain tubes and allowed to clot, the centrifuged to obtain serum using a bench to centrifuge.

Data and Statistical Analysis: Data was expressed as mean ± standard deviation of means. Collected data were analyzed using one way ANOVA in SPSS 10. Duncan's test was used in all data where appropriate. Probability level of < 0.05 indicates significant difference.

RESULTS

Table 1. Shows mean values of changes in body weight in the treatment and control groups. The Apple Vinegar had no effect on the weight of the studied animals. Apple Vinegar produced a dose-dependent hypoglycemia in normal rat's 7.44±0.93 mmol/L. It produced maximum reduction in blood glucose at the 14th day of experiment in comparison with normal control 7.74 ± 0.96 mmol/L (Table 2).

Dose-dependent reduction in blood glucose was also observed in alloxan-induced diabetic rats treated with Apple vinegar. The percent reduction in blood glucose tended to be higher in the diabetic condition compared to the normal state. A significant ($p< 0.05$) reduction in blood glucose of diabetic rats 8.3±0.75 mmol/L in case of administration of Apple Vinegar in comparison with diabetic control 24.6±8.45 mmol/L (Table 2). Glibenclamide drug (600µg/kg) produced a significant ($p< 0.05$) reduction in blood glucose 12.27 ± 4.77 mmol/L compared to control (Fig. 1).

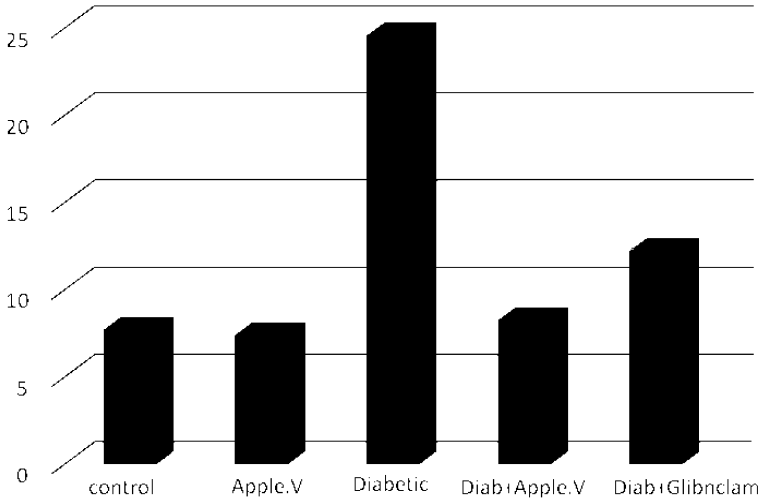

Fig. 1: Blood glucose concentration (mmol/L) of normal control, uncotrolled diabetic with Glibenclamide and apple vinegar treated diabetic rats

Table 1: Mean values of body weights of the different groups of rats (g)

Groups	Initial body weight	Body weight after 14 d of experiment
Normal control	221.4±14.92*	277.6±12.850
Normal+Apple vinegar	210.2±8.37	247.8±24.870
Diabetic control	205.8±8.34	231.2±11.420
Diabetic+Apple vinegar	167.6±6.05	172.33±26.92
Diabetic+Glibenclamide	182.2±3.76	207.8±36.910

*Values are given as mean ± S.D. for five rats in each group. Significant differences appeared between the groups ($p<0.05$)

Table 2: Blood glucose concentration (mmol/L) of normal and experimental animals

Groups	0 Day	7 days	14 days
Normal control	6.84±0.65	7.33±1.5*	7.74±0.96
Normal + Apple vinegar	8.00±1.93	7.80±1.3	7.44±0.93
Diabetic control	20.4±7.450	22.52±8.65	24.6±8.450
Diabetic+Apple Vinegar	10.30±2.75	9.78±1.98	8.3±0.750
Diabetic+Glibenclamide	13.26±3.77	12.88±2.97	12.27±4.77

*Values are given as mean ± S.D. for five rats in each group. Significant differences appeared between the groups ($p<0.05$)

DISCUSSION

Diabetes is growing epidemic around the world, which consider as chronic incurable condition due to insulin deficiency that affects 10% of the population [4]. Traditional plant medicines are used throughout the world for a range of diabetic complications. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future [6].

Apple Vinegar is used traditionally by diabetic patients in Jordan and is taken as different concentrations. Due to this reason the Apple Vinegar was evaluated and the data also confirmed the traditional indications. Studies by Brighenti *et al*. [13] and Ostman *et al*. [14] indicated the antidiabetic activity of the Apple Vinegar which substantiates the results of our studies in rats and rabbits. Moreover, the fact that the Apple Vinegar has a more prolonged effect than the glibenclamide dose after treatment indicates a prolonged duration of antidiabetic action and could be due to multiple sites of action possessed by the active principles of Apple Vinegar.

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia [11]. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species,

Alloxan and the product of its reduction, dial uric acid; establish a redox cycle with the formation of super oxide radicals. These radicals undergo dismutation to hydrogen peroxide [15]. Therefore, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of ß-cells [16].

It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan-induced diabetes whereas they are inactive in intense alloxan diabetes nearly all β-cells have been destroyed [17, 23].

Since the present results showed that the administration of apple vinegar and glibenclamide to diabetic rats restored the level of blood glucose. Alloxan-treated rats receiving the Apple Vinegar showed rapid normalization of blood glucose levels in comparison to control and this could be due to the possibility that some β-cells are still surviving to act upon by Apple Vinegar to exert its insulin releasing effect also glibenclamide reduced blood glucose levels in hyperglycemic animals, when state of diabetes is not severe. Moreover, oral administration of Apple Vinegar produced hypoglycemia in normal animals. This suggests that the mode of action of the active ingredients of Apple Vinegar is probably mediated by an enhanced secretion of insulin, like sulphonylureas [18, 19].

Several mechanisms to account for these effects have been proposed, including interference with enzymatic digestion of complex carbohydrates [20], delayed gastric emptying and enhanced peripheral glucose uptake and conversion to glycogen [9].

Acetic acid is the active ingredient in Apple vinegar, which has been suggested to explain vinegar's antiglycemic effects. Acetic acid may slow gastric emptying [21] alternatively, acetic acid may inhibit disaccharidase activity in the small intestine blocking the complete digestion of starch molecules or promote glucose uptake by muscle [10].

Study carried by Brighent *et al.* [13], showed that the insulin response curve was reduced after ingestion of sucrose 20%, when administered with Apple vinegar reduced the glycemic response to a mixed meal by more than 30%. Our results also demonstrated that the Apple Vinegar had a dose-response effect to reduce blood glucose concentration in normal and diabetic rats. The reduction in blood glucose noted in this trial is significant; however, any diet strategy that improves glycemic control is welcomed. Furthermore, the addition of Apple Vinegar to meal plans is simplistic, inexpensive and appetizing.

In conclusion, a significant antidiabetic activity with the apple vinegar and supports the traditional usage for activation of pancreatic β cells and control of diabetes. Hence it might help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of antidiabetic drugs.

ACKNOWLEDGMENTS

This study was supported by grant no. 27/10 from Al-Hussein Bin Talal University.

REFERENCES

1. Hamdan, I. and F. Afifi, 2004. Studies on the *in vitro* and *in vivo* hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. J. Ethnopharmacol., 93: 117-121.
2. Boden, G. and G. Shulman, 2002. Free fatty acids in obesity and type 2 diabetes:defining their role in the development of insulin resistance and beta-cell dysfunction. European Journal Clinical Investigation, 32: 14-23.
3. McGarry, J.D. and L. Banting, 2001. dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes, 51: 7-18.
4. Sangameswaran, B. and K. Ilango, 2010. Evaluation Anti-hyperglycemic and antihyperlipidaemic activities of Andrographis lineata Nees on Streptozotocin induced diabetic Rats. Jordan J. Biological Sci., 3: 83-86.
5. Sebbagh, N., C. Cruciani, F. Ouali, M. Berthault, C. Rouch, D. Chabane and C. Magnan, 2009. Comparative effects of Citrullus colocynthis, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats. Diabetes & Metabolism, 35: 178-184.
6. Srinivas, N., K. Murthy, D. Srinivas and S. Ravindra, 2003. The juice of fresh leaves of Catharanthus roseus Linn. Reduces blood glucose in normal and alloxan diabetic rabbits. BMC Complementary and Alternative Medicine, 3: 4-8.
7. Goldstein, L., 2000. Our four favorite healing vinegar remedies. Prevention J., 52: 255-257.
8. Johnston, C.S., C.M. Kim and A.J. Buller, 2004. Vinegar improves insulin sensitivity to a high-carbohydrate meal in subjects with insulin resistance or type 2 diabetes. Diabetes Care, 27: 281-283.

9. Arline, D., S. Carol, M. Buyukbese, D. Panayiotis and S. Mitchell, 2009. Vinegar lacks antiglycemic action on enteral carbohydrate absorption in human subjects. Nutrition Res., 29: 846-849.
10. Carol, S., M. Andrea and M. Shannon, 2009. Preliminary evidence that regular vinegar ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes mellitus. Diabetes Research and Clinical Practice, 84: 615-617.
11. Stanely, P., N. Kamalakkannan and P. Venugopal, 2004. Antidiabetic and antihyperlipidaemic effect of alcoholic Syzigium cumini seeds in alloxan induced diabetic albino rats. J. Ethnopharmacol., 91: 209-213.
12. Pari, L. and M. Uma, 1999. Hypoglycemic effect of Musa sapientum L. in alloxan-induced diabetic rats. J. Ethnopharmacol., 68: 321-325.
13. Brighenti, F., G. Castellani and L. Benini, 1995. Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. European J. Clinical Nutrition, 49: 242-249.
14. Ostman, E., Y. Granfeldt, L. Persson and I. Bjorck, 2005. Vinegar Supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects, European J. Clinical Nutrition, 59: 983-988.
15. Colca, J., N. Kotagel, C.L. Brooks, P. Lacy, M. Landt and M.L. McDanield, 1983. Alloxan inhibition of Ca2+ and calmodulin-dependent protein kinase in pancreatic islets, J. Biology and Chemistry, 225: 7260-63.
16. Mansi, K. and J. Lahham, 2008. Effect of Artemisia sieberi Besser (A. herba-alba) on heart rate and some hematological values in normal and alloxan-induced diabetic rats. J. Basic and Appl. Sci., 4: 57-62.
17. Grodsky, G.M., G.H. Epstein, R. Fanska and J.H. Karam, 1971. Pancreatic action of sulphonylureas. Fed. Proc., 36: 2719-28.
18. Sakanaka, S. and Y. Ishihara, 2008. Comparison of antioxidant properties of persimmon vinegar and some other commercial vinegar in radical-scavenging assays and on lipid oxidation in tuna homogenates. Food Chemistry, 107: 739-744.
19. Laura, L., H. Logan, C. Jerald and B. Morela, 2005. Esophageal Injury by Apple Cider Vinegar Tablets and Subsequent Evaluation of Products. J. American Diet Association, 105: 1141-1144.
20. Ogawa, N., H. Satsu and H. Watanabe, 2000. Acetic acid suppresses the increase in disaccharides activity that occurs during culture of caco-2 cells. J. Nutrition, 130: 507-13.
21. Johnston, C.S and A.J. Buller, 2005. Vinegar and peanut products as Complementary foods to reduce postprandial glycaemia, J. American Diet Association, 105: 1939-1942.
22. Ebihara, K. and A. Nakajima, 1988. Effect of acetic acid and vinegar on blood glucose and insulin responses to orally administered sucrose and starch. Agric. Biol. Chem., 52: 311-2.
23. Stanely, M.P., V.P. Menon and L. Pari, 1988a. Hypoglycaemic activity of Syzigium cumini seeds: effect on lipid peroxidation in alloxan diabetic rats. J. Ethnopharmacol., 61: 1-7.