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Efficacy of Diabetinol™ on glycemic control in insulin resistant hamsters and subjects with impaired fasting glucose – a pilot study

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ABSTRACT

The anti-diabetic potential of Diabetinol™ was tested in an animal model and in a pilot study in humans. Male Syrian golden hamsters ($n = 18$) were fed regular chow, 60% fructose or a 60% fructose diet + 1% Diabetinol™. Hamsters fed 60% fructose + 1% Diabetinol™ demonstrated a decrease in blood glucose, serum insulin, total cholesterol and triacylglycerol levels as compared to the fructose-fed animals. Efficacy of Diabetinol™ (2×525 mg/day) was investigated in a randomized, placebo-controlled, double-blind trial where subjects ($n = 19$) with impaired fasting glucose (IFG) on either Diabetinol™ or placebo were presented with a standard oral glucose challenge. After 84 days of supplementation a significant reduction ($p < 0.01$) in glucose intolerance, total cholesterol, LDL-cholesterol and decreasing trends in the HbA1c were observed in subjects supplemented with Diabetinol™. This study demonstrated an important future role for the Diabetinol™ in glycemic control and management of risk factors in subjects with IFG on oral medication.

1. Introduction

The increase in type II diabetes is a major health problem associated with complications that affect all age groups (Canadian Diabetes Association, 2008). The World Health Organization (2006) statistics have estimated 246 million people worldwide to be affected by diabetes. The incidence rates are at a further 7 million developing diabetes each year resulting in an estimated 389 million diabetics by 2025 (Zimmet et al., 2001).

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Abbreviations: CBFCs, citrus polymethoxylated flavones; HbA1c, haemoglobin A1c; WHO, World Health Organization; HDL, high density lipoproteins; IR, insulin resistance; OGTT, oral glucose tolerance test; AUC, area under curve; TZDs, thiazolidinediones; LDL, low density lipoproteins; EDTA, ethylene di-amine tetra acetic acid; NEFA, non-esterified fatty acids; SOPs, standard operating procedures; IRB, institutional review board; GCP, good clinical practices; TG, triacylglycerols; TRF, tocotrienol rich fraction; PMF, polymethoxy flavones; VLDL, very low-density lipoproteins; PPAR, peroxisome proliferator-activated receptor; VAS, visual analog scale; ATP III, adult treatment panel III; NCEP, National cholesterol education program; UKPDS, United Kingdom prospective diabetes study; EPIC-Norfolk, the European prospective investigation into cancer norfolk; CAD, coronary artery disease; AACE, the American association of clinical endocrinologists; CAM, complementary and alternative medicine; ACCORD, action to control cardiovascular risk in diabetes; ADVANCE, action in diabetes and vascular disease.

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Diabetes is described as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both and is defined by a fasting plasma glucose level of $\geqslant$7.0 mmol/L (American Diabetes Association, 2008). The United Kingdom prospective diabetes study (UKPDS) demonstrated that a progressive deterioration in beta cell function in the pancreas occurred over time and at clinical diagnosis of type II diabetes pancreatic islet function was only about 50% of normal (Wajchenberg, 2007). The UKPDS study also demonstrated that improved glycemic control may lower diabetes related complications and associated health care costs (Stevens et al., 2001).

The oral glucose tolerance test (OGTT) is recommended as a tool in diagnosis of impaired glucose tolerance and diabetes. By World Health Organization (WHO) definition, subjects with impaired glucose tolerance have blood glucose levels between 7.8 and 11.1 mmol/L, 120 min after an oral glucose load (Mannucci et al., 2003).

Current therapies in the prevention and treatment of type II diabetes include diet and medications targeting obesity, glucose levels, and more recently, insulin sensitizers, fibrates and thiazolidinediones (TZDs). Therapies involving existing pharmaceuticals have limited efficacy or tolerability and produce significant side effects (Moller, 2001).

During recent years nutraceutical products have been reported to reduce hyperglycemia and lipid disorders in individuals with type II diabetes or with predisposition to type II diabetes. Vuksan et al. (2000, 2001) reported on the effects of a ginseng supplement to reduce postprandial glycemia in healthy subjects and in individuals with type II diabetes. Others have demonstrated that blood glucose and insulin levels may be influenced by active ingredients from tea and coffee (Anderson & Polansky, 2002; Van Dam & Feskens, 2002). The potential role of dietary soy protein in modulation of diabetes has been investigated in two clinical trials with mixed results (Hermansen et al., 2001; Jayagopal et al., 2002). However there is a paucity of evidence from randomized placebo controlled clinical studies conducted for adequate periods of time in the management of type II diabetes.

The responses to dietary supplements containing citrus bioflavonoids in subjects with moderate hypercholesterolemia demonstrated that a 4-week treatment improved blood lipid profiles without causing any adverse effects (Kurowska et al., 2001). Although plasma glucose and insulin levels were not measured, the lipid-lowering responses appeared to be more pronounced in individuals with baseline characteristics consistent with metabolic syndrome. The combined results from experimental and clinical studies suggest that citrus bioflavonoids and tocotrienols may improve glycemic control and reduce postprandial glycemia. Other citrus bioflavonoids complex formulations have previously been reported to decrease plasma low-density lipoproteins in animal and human studies (LDL) (Kurowska et al., 2001; Kurowska et al., 2004). This action is speculated to occur *via* the inhibition of the synthesis of core protein Apo-B required for LDL synthesis in the liver (Borradaile et al., 1999). Whether Diabetinol™ through its inhibitory influence on Apo-B has an upward regulation effect on the insulin receptor sensitivity to insulin in type II diabetes remains to be clearly defined.

The objective of this study was to examine the efficacy and safety of Diabetinol™, on glycemic control in fructose-induced insulin resistant hamsters and in subjects with mild impaired fasting glucose (IFG).

2. Materials and methods

2.1. *Hamster study*

Eighteen male Syrian golden hamsters (*Mesocricetus auratus*, Charles River, Saint-constant, QC) were group housed and fed regular chow diet for seven days for acclimatization prior to the initiation of the experimental diets. Hamsters were maintained in a light/dark cycle of 12 h of light and 12 h of darkness, at a temperature between 18 and 26 °C and relative humidity between 30 and 70%. After acclimatization the hamsters were weighed and randomly assigned to one of three groups: A negative control group on regular chow diet ($n = 6$), positive control group on 60% fructose diet (Dyets Inc., No. 161506, Bethlehem, PA) ($n = 6$) or a 60% fructose + 1% Diabetinol™ treatment group ($n = 6$).

A 60% fructose diet is traditionally used for the induction of hypertriglyceridemia and insulin resistance in hamsters (Taghibiglou et al., 2000) The diet composition was as follows: casein (220 g), fructose (600 g), corn oil (60 g), cellulose fibre (70.9 g), L-arginine (1.0 g), L-tryptophan (1.1 g), salt/vitamin mixes (45 g) and choline bitartarate (2 g). There were no animal components in the diet and the diet was devoid of cholesterol.

The positive control and Diabetinol™ treatment group were fed a fructose-enriched diet for three weeks to induce hypertriglyceridemia and insulin resistance (Taghibiglou et al., 2000). During these three weeks hamster weight was monitored every two days and assessed at the end of three weeks for the development of insulin resistance by monitoring glucose, triacylglycerols, cholesterol, and insulin levels. After three weeks the positive control group of hamsters continued for an additional four weeks on the 60% fructose diet while the Diabetinol™ group of hamsters were fed the 60% fructose + 1% Diabetinol™ thus completing a total of seven weeks on the diets.

A clinical examination was performed on all animals at least once prior to initiation of treatment, and periodically during treatment and observations were recorded. Routine cage-side observations were made on all animals throughout the study for general signs of pharmacologic effects, morbidity and mortality. The hamsters were fed *ad libitum*, weights monitored once every week and food consumption measured once a week over a 24 h period.

At randomization (baseline/day 0), and day 21, three weeks prior to start of Diabetinol™, blood samples were collected from the retro-orbital venous plexus from hamsters deprived of food for 16 h for the measurement of glucose, insulin, triacylglycerols, total cholesterol, and non-esterified fatty acids (NEFA). Hamsters were sacrificed on day 49 (end of study) and blood samples were collected by cardiac puncture for the measurement of glucose, insulin, triacylglycerols, total cholesterol, non-esterified fatty acids (NEFA).

All animal procedures were executed in a manner to avoid animal suffering or pain. Procedures for this study were

approved by the Animal Care and Use Committee at The University of Western Ontario (UWO) and were performed according to guidelines of the Canadian Council on Animal Care and applicable UWO, London Health Sciences Centre (LHSC) and KGK Synergize standard operating procedures.

2.1.1. *Analytical procedures*

Glucose was determined on whole blood using a glucometer and serum was separated after centrifugation at 3000*g* for 20 min at 4 °C and stored at −80 °C until analyzed for insulin, triacylglycerols and cholesterol. Insulin was measured using specific enzyme-linked immunosorbent assays (ELISA; Linco Research, St. Charles, MO, USA), and triacylglycerols, total cholesterol and non-esterified fatty acid (NEFA) levels were determined by enzymatic colorimetric assays using commercially available kits (Wako, R1-Cat # 998-40391, R2-Cat # 994-40491 Wako, Cat # 439-17501 and Wako Cat # 994-75409, respectively, Wako Diagnostics, Richmond, VA).

2.1.2. *Statistical analysis*

Statistical analysis of mean body weights (g), estimated food consumption (g/kg body weight/day), insulin, blood glucose, total cholesterol and triacylglycerols within and between study groups were determined using ANOVA followed by Holm-Sidak multiple comparisons test. Probability values $p \leqslant 0.05$ were accepted as significant.

2.2. *Human pilot study*

2.2.1. *Subjects and design*

The study was a randomized, double-blind, placebo-controlled study conducted at SIBR Research, Inc., West Bradenton, Florida, USA. Nineteen subjects with characteristics of metabolic syndrome and on oral hypoglycemic medications were screened and evaluated for inclusion in the study from existing patient databases or by advertisement. To qualify for entry into the study subjects had to be between BMI 25 and 39.9 kg/m^2, weight stable (for 3 months prior to study), 18–75 years old, have a fasting glucose level between 6.1 and 9.0 mmol/L (109.8–162 mg/dL), and a HbA1c level ⩽7%. Subjects were required to discontinue other natural health products three weeks prior to randomization and during the study and to maintain their current level of physical activity and dietary habits during the course of the study.

Subjects were excluded from participating if they were diabetic and required insulin therapy, pregnant, breastfeeding, or planning to become pregnant, had participated in a clinical research trial within 30 days prior to randomization, on natural health products other than multivitamin and mineral supplements, on prescribed medication or over-the-counter supplements for weight loss within the previous three months, had an allergy or sensitivity to study supplement ingredients or had any other condition which in the Investigator's opinion may have adversely affected the subject's ability to complete the study or its measures.

This study was conducted in accordance with good clinical practice guidelines and the ethical principles of the Declaration of Helsinki (2000). The study protocol and materials were approved by the IntegReview Ethical Review Board (Austin, TX), and all subjects gave written informed consent prior to participation.

2.2.2. *Study protocol*

The study volunteers were randomized in a 1:1 ratio into two groups one of which received Diabetinol™ 525 mg and the other a placebo (cellulose, 525 mg/day) twice daily for a period of twelve weeks. The study included 5 clinic visits, which occurred at screening, baseline (day 0), day 28, 56 and 84.

At screening, subjects provided written informed consent, reviewed inclusion and exclusion criteria, and detailed their medical history and their prior use of concomitant medications. Heart rate, blood pressure, height, weight, hip and waist circumference were measured and BMI calculated. Fasting peripheral blood was collected for the determination of serum chemistry, hematology, glucose, HbA1c, and lipid profile. Further, subjects provided a urine sample for pregnancy testing.

At baseline or randomization and at all other visits blood pressure and heart rate were assessed, anthropometric measurements recorded, BMI, waist/hip ratio were calculated and pregnancy testing conducted if appropriate. Fasting blood was collected for the determination of glucose, insulin and HbA1c. An oral glucose tolerance test (OGTT), where subjects consumed 100 g of a glucose beverage over a 10 min period test was conducted on all subjects at baseline, day 28, 56 and 84 and blood samples collected at 30, 60, 120, 180 and 240 min post glucose consumption for analysis of glucose and insulin.

Dispensed at baseline, day 28 and 56, the treatment diary included forms to record daily product use, changes in concomitant therapies and adverse events. On day 28, 56 and 84, subject treatment diary was returned and reviewed, compliance calculated and adverse events were recorded. On day 84, prior to the OGTT an additional 10 ml of blood was collected for serum chemistry, hematology and for the determination of the lipid profile. Safety evaluations included vital sign measurements, laboratory test results and adverse event reporting.

2.2.3. *Treatments*

Diabetinol™, was encapsulated by Innovative Health Products, Largo, Florida. Diabetinol™ a >62% polymethoxylated flavone extract was composed mainly of 49% nobiletin and 13% tangeretin. Placebo capsules were filled with cellulose. Both test product and placebo were stored at room temperature between 17 and 25 °C. Subjects were instructed to take two capsules per day (525 mg per capsule) with food, in the morning and in the evening. Test article was dispensed at baseline, day 28 and 56 and returned at day 28, 56 and 84.

2.2.4. *Statistical analysis*

No formal sample size calculation was done for this study as it was a pilot trial. Group descriptive statistics were calculated for each study group. Fasting glucose, insulin, HbA1c and AUC glucose and insulin were compared within group and HbA1c levels between the two groups by Student's *t*-test. Statistical significance was established at $p < 0.05$.

3. Results

3.1. Hamster study

Hamsters in all groups showed an increase in body weight (g) from baseline to day 49. Animals fed regular chow (control group) showed the highest weight gain (127.7 ± 5.9g day 0 to 158.9 ± 12.1g day 49) while those in the Diabetinol™ group showed least weight gain (125.7 ± 4.9g day 0 to 144.1 ± 7.3g day 49). At the end of the study (day 49), Diabetinol™-treated hamsters showed decreased glucose (Fig. 1) and insulin levels as compared to hamsters in the 60% fructose fed positive control group. It was seen that increase in insulin values from day 0 correlated with the increase in glucose levels (Table 1). There was a significant difference in triacylglycerol levels in chow-control and fructose-control hamsters ($p < 0.05$) on day 49. Hamsters consuming Diabetinol™ demonstrated lower levels of triacylglycerols in comparison to fructose fed hamsters at all time points. Hamsters in all groups showed significant increases in cholesterol from day 0 to day 21 ($p < 0.05$). However those fed Diabetinol™ demonstrated a decrease in cholesterol levels from day 21 to day 49, while those in the other groups continued to show an increase in cholesterol.

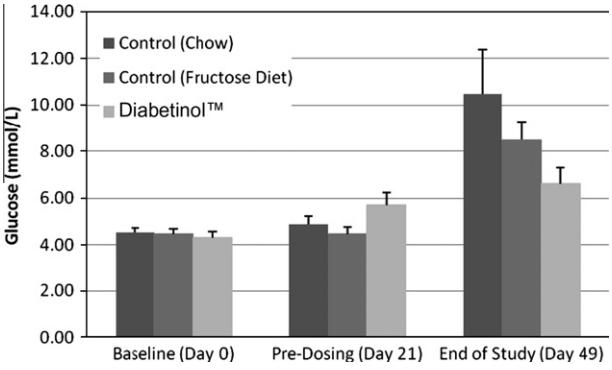

Fig. 1 – Glucose levels in placebo- and Diabetinol™-treated hamsters at baseline, prior to treatment and at study completion. Statistical analysis of blood glucose was determined using ANOVA followed by Holm-Sidak multiple comparisons test. Each data point is represented as mean ± SEM.

3.2. Human study

Analysis of baseline subject demographics demonstrated that both study groups were similar except for a significant ($p < 0.05$) difference in group mean weight (Diabetinol™, 95.85 ± 6.78 kg, and placebo, 106.05 ± 8.82 kg) and group mean age (Diabetinol™, 60.33 ± 2.26 years, and placebo, 54.20 ± 3.81 years). The baseline fasting glucose was not different between groups (Diabetinol™ 130.33 ± 3.64 mg/dL and placebo 129.80 ± 8.52 mg/dL) but was above acceptable levels (<100 mg/dL) and indicated that subjects in both groups had mild hyperglycemia (100–150 mg/dL). Baseline HbA1c levels were not significantly different between subjects on placebo and Diabetinol™ (6.53 ± 0.22% and 6.39 ± 0.22%, respectively).

Subjects supplemented with Diabetinol™ or placebo demonstrated a significant increase in plasma glucose levels from 0 min to 120 min during the glucose challenge ($p < 0.01$). The plasma glucose of subjects in both groups returned to baseline levels after 240 min. At baseline, 28, 56 and 84 days the peak in the plasma glucose curve occurred between 60 and 120 min in subjects on Diabetinol™ or placebo (Fig. 2a and b). After supplementation for 56 and 84 days the glucose response curve of subjects on the Diabetinol™ group was blunted as compared to the levels in the baseline OGTT curve. Further, after 84 days of supplementation subjects on

Table 1 – Biomarkers in control animals and Diabetinol™ treated animals at day 0, prior to treatment (day 21) and at study completion (day 49).

	Control (chow) ($n = 6$) [N] Mean ± SEM	Control (fructose diet) ($n = 6$) [N] Mean ± SEM	Diabetinol™ (1% in diet) ($n = 6$) [N] Mean ± SEM
Glucose (mmol/L)			
Day 0	[6] 4.5333 ± 0.1820	[6] 4.4667 ± 0.2445	[6] 4.3000 ± 0.2875
Day 21	[6] 4.8833 ± 0.3572	[6] 4.4667 ± 0.3018[a]	[6] 5.7167 ± 0.5282
Day 49	[6] 10.467 ± 1.9043	[6] 8.4833 ± 0.7833[b]	[6] 6.6000 ± 0.6952
Insulin (ng/mL)			
Day 0	[6] 1.4500 ± 0.0480	[6] 1.4767 ± 0.0599	[6] 1.5750 ± 0.1628
Day 21	[6] 1.3650 ± 0.0710[a]	[6] 1.4000 ± 0.1171[a]	[5] 1.5080 ± 0.2777[a]
Day 49	[6] 3.6200 ± 0.6949[b]	[6] 3.7467 ± 0.5599[b]	[6] 3.3050 ± 0.6017[b]
Cholesterol (mg/dL)			
Day 0	[6] 131.01 ± 4.9491[a]	[6] 123.73 ± 6.2644[a]	[6] 131.64 ± 8.8919[a]
Day 21	[6] 207.41 ± 15.497[b]	[6] 212.33 ± 14.769[b]	[6] 225.20 ± 11.910[b]
Day 49	[6] 210.03 ± 12.188	[6] 224.77 ± 21.251	[6] 194.71 ± 24.563
Triacylglycerols (mg/dL)			
Day 0	[6] 116.38 ± 10.089	[5] 104.72 ± 19.359	[6] 67.142 ± 7.2517
Day 21	[6] 104.20 ± 14.493	[6] 174.50 ± 49.560	[6] 122.91 ± 26.460
Day 49	[6] 96.985 ± 14.778[a]	[6] 210.23 ± 46.589[b]	[6] 171.44 ± 24.085

Different superscripts in the same column and same row are significantly different by ANOVA followed by Holm-Sidak multiple comparisons test. a vs. b, $p < 0.05$.

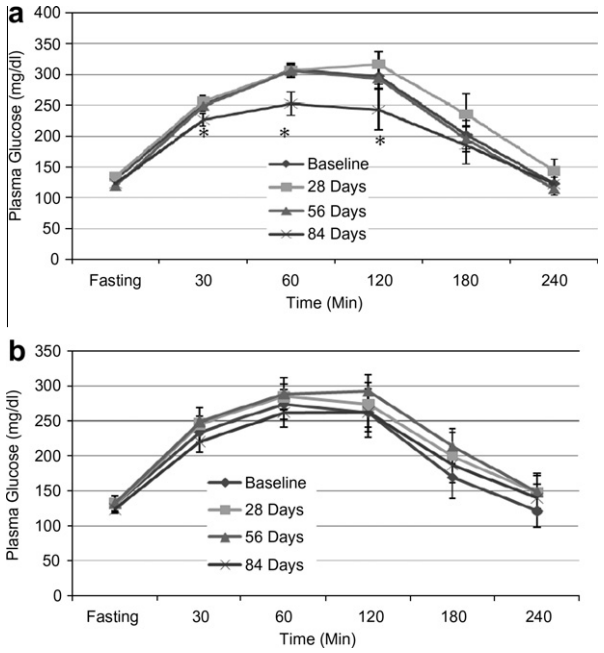

Fig. 2 – Plasma glucose response curves to a standard glucose tolerance test before and after 28, 56 and 84 days of (a) *Diabetinol™ and (b) placebo supplementation. *The plasma response curve at 84 days was significantly different ($p < 0.01$) from baseline at 30, 60 and 120 min by Student *t*-test. Each data point is represented as mean ± SEM.

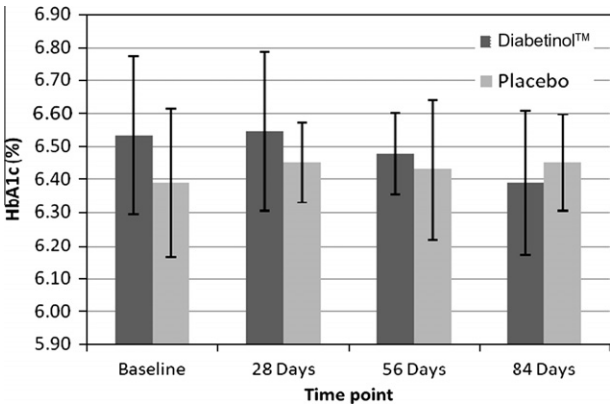

Fig. 3 – Plasma HbAlc levels for placebo and Diabetinol™ after 28, 56 and 84 days of supplementation. Statistical analysis was determined using Student *t*-test. Each data point is represented as mean ± SEM.

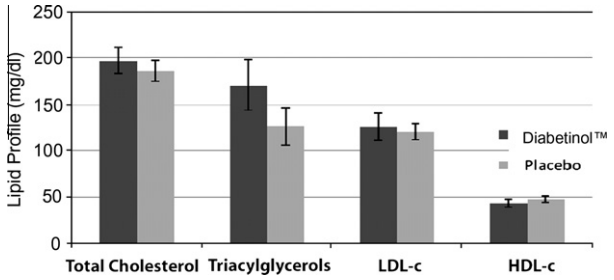

Fig. 4 – Baseline plasma lipids for placebo and Diabetinol™. Statistical analysis of plasma lipids were determined using Student *t*-test. Data points are represented as mean ± SEM.

Diabetinol™ demonstrated a significant reduction in plasma glucose at 30, 60 and 120 min after the glucose challenge when compared to the plasma glucose levels after the OGTT at baseline ($p < 0.01$). The plasma glucose response curves after a glucose challenge at 28, 56 and 84 days for subjects on the placebo were not significantly different from that of their baseline values (Fig. 2b).

There was no significant difference in mean fasting glucose values at baseline or day 84 between groups, Diabetinol™ (from 130.33 ± 3.64 mg/dL at baseline to 123.33 ± 6.70 mg/dL) or placebo (129 ± 8.52 to 123.90 ± 5.04 mg/dL) (Fig. 2a and b).

There was an increase of 9.0 mg/dL in mean plasma glucose on day 28 and a reduction of 9.0 mg/dL on Day 56, and a significant reduction of 56.0 mg/dL ($p < 0.01$) on day 84 from baseline in subjects on Diabetinol™ compared to those on placebo (data not shown).

A mean reduction in AUC of 127 mg/dL/h from baseline to day 84 was demonstrated in the Diabetinol™ group while there was a 10 mg/dL/h increase in subjects in the placebo group (data not shown).

There was no difference in plasma HbA1c levels between placebo and Diabetinol™ groups at baseline, day 28, 56 or 84 (Fig. 3). After 84 days of supplementation decreasing trends in the HbA1c were observed in subjects supplemented with Diabetinol™ compared to subjects on placebo. Fasting plasma insulin levels were not significantly different between subjects on placebo and Diabetinol™ at baseline, day 28, 56 or 84 and did not change significantly from baseline to day 28, 56 or 84 in Diabetinol™ or placebo (data not shown).

There were no significant differences between subjects in placebo and the Diabetinol™ at baseline for total plasma cholesterol, triacylglycerols, LDL-cholesterol and HDL-cholesterol levels (Fig. 4). This cohort of subjects had lipid levels which were higher than the clinically acceptable range for these parameters. Subjects in the Diabetinol™ group demonstrated a 13.29% reduction in total cholesterol ($p < 0.01$) and a 22.79% reduction in LDL-cholesterol ($p < 0.01$) from baseline to 84 days (Fig. 5a). There were no significant changes in the lipid profiles from baseline to day 84 in subjects on placebo (Fig. 5b).

3.2.1. *Safety and adverse events*

Baseline hematology was in the normal and acceptable range and not significantly different between subjects in either the Diabetinol™ or the placebo group. After 84 days of treatment the mean platelet count increased significantly ($p < 0.05$) in the placebo group and mean haemoglobin decreased significantly ($p < 0.05$) in the Diabetinol™ group (data not shown). Although these changes were significant values were still within normal and clinically acceptable limits. There were no significant differences in blood pressure, pulse rate or any other vital signs in subjects on Diabetinol™ or placebo.

A total of 9 adverse events were reported by subjects on Diabetinol™ and a total of 8 by those on placebo. The adverse events reported by subjects on Diabetinol™ were diarrhea (4),

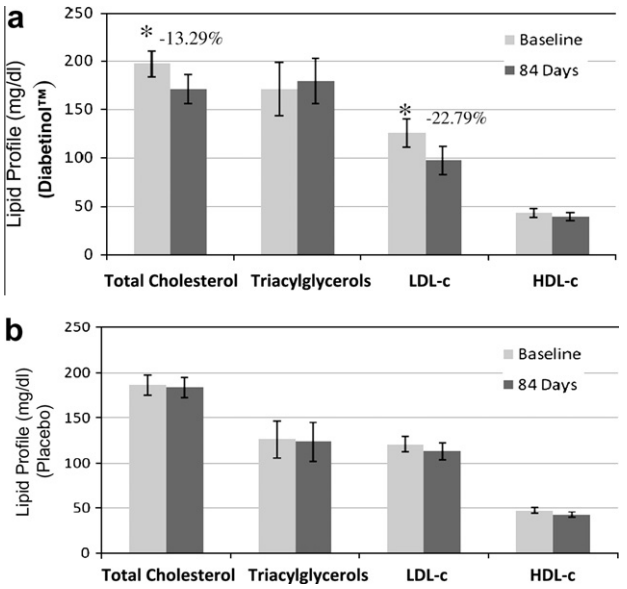

Fig. 5 – (a) *Diabetinol™ group plasma lipids levels after 84 days of supplementation compared to the baseline (day 0) levels (b) placebo control group plasma lipids levels after 84 days of supplementation compared to the baseline(day 0) levels. *$p < 0.01$, day 84 values are significantly different from baseline values by Student *t*-test. Each data point is represented as mean ± SEM.

constipation (1), increase in appetite (1), rash (1), nausea (1), heartburn (1), insomnia (1), fatigue (1), excess thirst (1), and body pain (1). Subjects on placebo reported constipation (1), nausea (1), heartburn (1), excess thirst (1), edema (1), hypoglycemia (1), bad dreams (1) and flatulence (1). The diarrhea reported by four subjects on Diabetinol™ was resolved within one month from start of treatment.

4. Discussion

Evidence exists that interventions can reduce the progression of impaired glucose tolerance to diabetes mellitus over time, and this evidence has also driven the recommendation to promote formal glucose tolerance testing over a wider range of fasting plasma glucose levels than impaired fasting glucose alone (Tuomilehto et al., 2001). Impaired glucose tolerance is a phase between "at risk" and the "development of diabetes". It is reasonable to assume that "at risk" patients with normal glucose tolerance or impaired fasting glucose would benefit from lifestyle interventions, and the need for definitive determination of impaired glucose tolerance is a must (Knowler et al., 2002). Using OGTT as a scoring system is of value as it provides guidance to researchers in modifying interventions that may already be proven to prevent the onset of diabetes.

Many pharmaceuticals are in place for the management of type 2 diabetes however the ACCORD (Action to control cardiovascular risk in diabetes) and the ADVANCE collaborative group study (Action in Diabetes and Vascular Disease,) have reported on their limitations in protection against cardiovascular risk (Gerstein et al., 2008; Patel et al., 2008). A meta-analysis of observational studies on combination therapy with metformin and glyburide was found to increase the risk of cardiovascular events and mortality (Rao et al., 2008). Other pharmaceuticals such as thiazolidinediones have been called into question because of an associated increase in cardiovascular and fracture risks (Lipscombe, 2009).

Use of complementary and alternative therapies medicine (CAM) have gained in popularity as consumers seek alternatives to conventional medications (Nahas & Moher, 2009). However, a recent review of the literature found a paucity of evidence on the evaluation of biomarkers that are a physician's target in treating diabetes (Nahas & Moher, 2009). These authors found that reasonable conclusions as to the efficaciousness of the intervention were limited due to factors such as inadequate study duration, poor design, confusing dose regimens and neglected addressing biomarkers that were of value to the clinician. Currently there is no CAM research addressing microvascular or macrovascular clinical outcomes of these therapies.

In animal models citrus bioflavonoids have been positively correlated with decreased cholesterol and an anti-diabetic effect. Previous studies on the effects of CBFC's on inflammation, insulin resistance and dyslipidemia have shown that these compounds down regulate the inflammatory response, prevent atherosclerosis and improve insulin resistance (IR) both *in vitro* and *in vivo* (Kurowska & Manthey, 2002; Kurowska et al., 2004; Whitman et al., 2005). In the current study, the anti-diabetic effect of Diabetinol™ was explored using a prediabetic hamster model of fructose-induced hyperlipidemia and IR (Taghibiglou et al., 2000). After treatment with Diabetinol™ for four weeks, hamsters on high-fructose diet demonstrated decreased glucose and insulin levels as well as reduced blood cholesterol, triacylglycerol and non-esterified fatty acid (NEFA) concentrations compared to hamsters receiving the positive control fructose diet. These results confirm the results of previous studies in a hamster model of fructose-induced dyslipidemic insulin resistance where treatment with PMF'S significantly reduced fructose-induced increases in serum cholesterol and NEFA, and significantly reduced TG concentration (Li et al., 2006; Kurowska & Manthey, 2002, 2004). The decreased glucose and insulin result correlates with the results of a previous study, on IR hamsters where treatment with 1% mixed PMF reduced glucose tolerance (Kurowska & Guthrie, 2003). A combination of 1% PMF and 1% TRF added to the casein-based diet induced a substantial reduction in fasting glucose concentration and improved glucose tolerance (Kurowska et al., 2003).

In the current human pilot study, subjects with impaired fasting glucose demonstrated a significant increase ($p < 0.01$) in glucose tolerance to a standardized glucose challenge, a significant decrease ($p < 0.01$) in fasting plasma LDL and demonstrated decreasing trends in the HbA1c levels after 84 days of supplementation. A statistically significant ($p < 0.01$) reduction in the peak hyperglycemic response in the Diabetinol™ group by 56 mg/dL indicated a noteworthy improvement in glucose tolerance when compared to the subjects on placebo.

Changes in HbA1c values are most often used to evaluate the glycemic control as HbA1c is an indicator of average blood glucose control over a three-month period and is correlated to an individual's risk of developing both micro-vascular (retinopathy, nephropathy and neuropathy) and macrovascular

(myocardial infarction and stroke) complications associated with diabetes (LeRoith, 2008). Both study groups appeared to be well controlled by their oral hypoglycemic medications as judged by the high normal HbA1c. A decreasing trend in HbA1c is important and is a measure of glucose management in type II diabetics over a 90-day interval (Wu, 2005).

The UKPDS study demonstrated that for every 1% point reduction in HbA1c, there was a 14 % reduction in the risk of myocardial infarction, a 21% reduction in diabetes-related deaths, a 12% reduction in stroke, and a 16% reduction in congestive heart failure (Wajchenberg, 2007). The significant improvement in glucose tolerance that was demonstrated in the subjects following the Diabetinol™ consumption was possibly associated in improving the HbA1c. These results were further confirmed with a significant ($p < 0.01$) reduction in plasma LDL in the subjects supplemented with Diabetinol™.

The results of this pilot study demonstrated that supplementation with the Diabetinol™ was safe and significantly blunted the glucose response during an oral glucose challenge in subjects with impaired fasting glucose. It is noteworthy that all subjects were on oral medications for the control of their diabetes and that supplementation with Diabetinol™ for 3 months provided significantly improved clinically important biomarkers (2 h post prandial glucose, HbA1c and improved LDL-cholesterol and TC). It is possible to suggest that Diabetinol™ may help lower blood glucose levels and be beneficial in lowering the risks of heart disease and diabetic complications in people with impaired fasting glucose.

5. Conclusions

In this study the efficacy and safety of Diabetinol™ components have been evaluated using both an *in vivo* and a human clinical study. The results of this study demonstrated that Diabetinol™ did not produce any adverse effects in a fructose-induced hamster model and showed improvement in blood glucose, insulin, cholesterol and triacylglycerols. The use of Diabetinol™ over a three-month interval in c subjects with IFG on oral medication was safe and significantly reduced the glucose intolerance measured as peak changes in blood glucose over the four hours of a standard glucose challenge and reduced plasma LDL levels. These data suggest that Diabetinol™ as a natural food product may have a protective effect in individuals with a combined IFG and hyperlipidemia. An additional six-month study is underway to evaluate Diabetinol™ treatment in a larger sample of subjects with type II diabetes.

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