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Research Article

Effect of Standardized Isoflavones Rich Soya Seed Extract on Glucose Utilization and Endurance Capacity in Type- II Diabetic Mice

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Abstract

Soya (*Glycine max* (L) Merr) isoflavones have the property to enhance mitochondrial biogenesis and improve the status of diabetes mellitus. Isoflavones rich soya seed extract (DRE) was standardized for daidzin using HPLC. In the present investigation, DRE (16.22 % w/w daidzin) was tested for its endurance capacity and glucose utilization in high fat diet and multiple low dose streptozotocin induced type-2 diabetic mice. DRE (140 mg/kg, p.o.), Metformin (10 mg/kg, p.o.) treatment and exercise were induced for a period of 28 days. The biochemical parameters, endurance capacity, glucose tolerance, utilization and muscle antioxidant level were measured at terminal. All the parameters were compared with diabetic control. DRE has showed significant ($p<0.01$) improvement in endurance capacity and metabolic flexibility which was reflected in improved muscle performance, glucose utilization in oral glucose tolerance test and insulin tolerance test. However, the exercise induced mice showed the best improvement in the endurance performance. Marked improvement in fasting plasma glucose, TG and TC was observed in DRE treated mice in comparison to diabetic control. Muscle antioxidant activity was measured by estimating SOD, catalase and MDA levels. DRE and Metformin treatment have showed significant ($p<0.01$) free radical scavenging activity compared to diabetic control. Moreover, the animals treated with DRE and Metformin combination demonstrated more potent activity in OGTT, ITT, fasting plasma glucose, TG level. Similar effect was also observed in skeletal muscle catalase and MDA activity. DRE treatment has significantly improved diabetic condition however, the combination of DRE and Metformin showed better efficacy and can be a potential therapeutic option.

ABBREVIATIONS

NIDDM: Non Insulin Dependent Diabetes Mellitus; T2D: Type 2 Diabetes Mellitus; PPAR: Peroxisome Proliferator - Activated Receptor; PGC: Peroxisome proliferator-activated receptor γ coactivator; SIRT1: Sirtuin (Silent mating type information regulation 2 homolog)1; CPSCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; DRE: Standardized isoflavones rich soya seed extract; STZ: Streptozotocin; TG: Triglyceride; TC: Total Cholesterol; OGTT: Oral Glucose Tolerance Test; ITT: Insulin Tolerance Test; SOD: Super Oxide Dismutase; MDA: Malondialdehyde; Met: Metformin; HFD: High Fat Diet; MLDS: Multiple Low Dose of STZ; ROS: Reactive Oxygen Species; AGEs: Advanced Glycosylation End products.

INTRODUCTION

Type II Diabetes mellitus (T2D) (NIDDM) is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Diabetes is often initially managed by increasing exercise and dietary modification. As the condition progresses, medications may be needed. T2D is primarily due to lifestyle factors and genetics [1]. A number of lifestyle factors are known to be important to the development of T2D. In one study, those who had high levels of physical activity, a healthy diet, did not smoke, and consumed

alcohol in moderation had an 82% lower rate of diabetes. When a normal weight was included the rate was 89% lower. Obesity has been found to contribute to approximately 55% type II diabetes. Decreasing the consumption of saturated fats and trans fatty acids while replacing them with unsaturated fats may decrease the risk. The increased rate of childhood obesity in between the 1960s and 2000s is believed to have lead to the increase in T2D in children and adolescents [1]. The number of people with diabetes in the world is expected to approximately double between 2000 and 2030, based solely upon demographic changes. The greatest relative increases will occur in the Middle Eastern, Crescent, sub-Saharan Africa, and India. India, china and USA will contribute most diabetic population of 79.4, 42.3 and 30.3 million respectively by 2025 [2]. The undesirable side effects of drugs currently used for the treatment of T2D and the limited access to public health systems in low-income communities motivate patients to use alternative therapies to counteract the complications associated with this disorder. Medicinal plants constitute a common alternative treatment for T2D in many parts of the world and it has been already postulated that a diet enriched with soya supplements may be beneficial for the prevention of T2D. The objective of the study is to examine the association between soya isoflavones and T2D.

The beneficial effects soy beans on glycemic control was first published in 1910 [3]. Until recently, there have been few studies of the effects of soy or its isoflavones on glycemic indices. These few studies did not assess potential mechanisms involved with changes in insulin action or whether the effect is due to the soy protein, its isoflavones, or both. However as per some studies the soy isoflavones (daidzein and genistein) bind to PPARγ as well as PPARα and δ suggesting the potential property of isoflavones as a nutritional approach to modulating insulin action [4]. Further, it has been reported that dietary soy isoflavones can prevent the development of diabetic cataracts and increase the insulin secretion in STZ induced diabetic rats [5]. It has also been demonstrated that, soya isoflavones (daidzein and geninstein) has increased the SIRT1 activity in kidney for mitochondrial biogenesis [6,7]. Although the effect of isoflavones was not studied in muscle mitochondria, the approach to increase the muscle mitochondrial biogenesis could have therapeutic value in diabetic patients. On increased mitochondrial activity, this will lead to improvement in muscle activity which will demand more glucose and thus the glucose utilization will increase. The enhanced mitochondrial activity will also result in increased endurance capacity of an individual. In our previous study, we have demonstrated the effect of soya isoflavones rich extract, metformin and exercise on metabolic status of STZ-Nicotinamide induced T2D rats [8]. There was significant improvement in biochemical parameters of diabetic rats treated with isoflavones rich extract+Metformine and exercise+Metformine. The endurance performance was also increased in isoflavones rich extract and exercise group [8]. Hence, the beneficial effect of isoflavones in terms of enhanced glucose tolerance, endurance capacity and metabolic flexibility could lead to improvement of health status and life style of obese - diabetic patients.

With this background information, we hypothesize that soy isoflavones rich in daidzin could be an add-on therapy for controlling the diabetic status and also could improve the life style of a diabetic patient. Therefore, this study was undertaken to evaluate the activity of standardized isoflavones rich extract on glucose utilization, endurance capacity of T2D mice.

MATERIALS AND METHODS

Plant material

The seeds of *Glycine max* were collected from the local market of Berhampur, Odisha (India) during the month of January, 2011. The seed specimen was authenticated in the P.G. Department of Botany and Biotechnology, Khallikote Autonomous College, Berhampur, Odisha (India).

Chemicals

Streptozotocin (STZ) was obtained from Himedia laboratories Pvt. Ltd. Mumbai, India, Metformin from Yarrow Chemical Products, Mumbai, India. Insulin from Torrent Pharmaceuticals Ltd.,India. Tris base and EDTA from Merk Ltd., Mumbai, India. Daidzin was obtained from Sigma Aldrich, USA. Biochemical kits for glucose, triglycerides and cholesterol estimation were obtained from Coral Clinical Systems, India. Solvents and reagents used were of analytical grade and obtained from Merk Ltd., Mumbai, India.

Animals and treatment

Sixty male Swiss albino mice (4 weeks of age, 10 - 15 g) were selected from the stock of animal house (Regd. No. 926/ab/06/CPSCSEA, 22-02-2006) of Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India. The animals were kept in polystyrene cages under standard laboratory conditions i.e. at temperature of 25 ± 1ºC, relative humidity of 60±2% and were exposed to a 12h photoperiod. The animals were fed normal chaw pellet (Rayan's biotechnologies Pvt. Ltd. Hyderabad, India) and water *ad libitum*. After one week of acclimatization, the protocol of diabetic induction and drug treatment was followed as described elsewhere in the text. The Institutional Animal Ethical Committee has approved the experimental protocol (Approval No.62/16.03.2011) prior to carry out the animal experimentation. Ten mice were kept in non-diabetic control group. Diabetes was induced in rest of 50 mice. The experimental groups are as below.

Group 1: Non-diabetic control fed with normal chaw diet and treated with normal saline daily.

Group 2: Diabetic control, 1% sodium CMC was administered daily.

Group 3: Diabetic, treated with 140 mg/kg of standardized isoflavones rich soya seed extract (DRE) daily.

Group 4: Diabetic, treated with 140 mg/kg of DRE combined with metformin (Met) 10 mg/kg daily.

Group 5: Diabetic, treated with 1% sodium CMC daily and exercised thrice a week.

Group 6: Diabetic, treated with 10 mg/kg Met daily.

Each group consists of ten animals. At the end of week 4, plasma glucose, TG, TC, OGTT, ITT, and muscle performance/endurance capacity was measured. SOD, catalase and MDA activity was estimated in gastrocnemious muscle of mice.

Extraction and complexation

The soy seeds were powdered and passed through mesh (#16). The powder was macerated with 99% methanol for 24h and then soxhleted for 36h. Crude extract was obtained and the isoflavones rich extract was concentrated from this methanol crude extract by complexation technique [9]. Briefly the methanol crude extract was treated with copper sulfate at 40°C for 4h. The residue was filtered out, washed with methanol and dried under vacuum to give residue R_1. The filtrate was concentrated to dryness, washed with acetone and dried to give a solid residue R_2. Both residues were hydrolyzed using dilute acid (10% HCl), to release free isoflavones which was extracted using ethyl acetate. The ethyl acetate extract was evaporated to dryness to give isoflavones rich fraction containing daidzin and genistein.

Standardization of Daidzin in isoflavones rich fraction of soya seed extract

DRE was analyzed by HPLC system (Schimadzu, Japan). The system category was class VP, using Lichrospher ® 100 column with RP_18 (5 µm), using mobile phase as water: acetonitrile (flow rate of 1.0 ml/min by gradient elution method). The retention time of daidzin was measured at λ_{max} 260 nm by using UV detector. The method was standardized and validated with an initial sampling of 10 µg/ml. Eight replicates of this sample was prepared and analysed. Limit of detection and limit of quantification was obtained as 5.01 and 10.34 µg/ml. Calibration curve was prepared using 10, 100, 300 and 500 µg/ml of daidzin. The curve showed good linearity with r^2 value of 0.998. DRE (16.22% w/w daidzin) was used for the pharmacological evaluation.

Induction of type II diabetes in mice

After one week of acclimatization, the mice were divided into five groups. The animals were fed with a high fat diet for 28 days. The basic composition of high fat diet was as follow (g/ 100 g high fat diet): Casein 28.9%, DL- Methionine 0.33%, corn starch 20.73%, sucrose 9.05%, vegetable hydrogenated oil 27.41%, corn oil 1.6%, cellulose 2.66%, vitamin and mineral mix 3%, calcium carbonate 0.67%. The drugs were solubilised in 1% CMC and administered by oral gavages between 11.00–12.00 h from day 14 to day 28. On day 15, the animals were injected with low dose of STZ (40 mg/kg, i.p.) dissolved in citrate buffer (pH 4.5) for five consecutive days. The mice were kept on the above treatments and fed with the high-fat diet until day 28. A separate group of animals were fed a normal chow diet and did not receive STZ injections (non-diabetic control) [10].

Collection of blood and determination biological parameters

Animals were fasted overnight for a period of 18 h. Blood (0.5 ml) was withdrawn from the retro-orbital sinus under mild anesthesia and was collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 10 min to obtain clear plasma [11]. The plasma was then analyzed for glucose, TG and TC in the biochemical analyser (3000 Evolution, BSI, Italy) using commercially available biochemical kits.

Oral glucose tolerance test (OGTT)

OGTT was performed in overnight (18 h) fasted mice according to the method described by Bonner-Weir (1988) [12]. The mice were allowed to drink water and the treatment groups were administered the respective drugs at the regular time. Initial plasma glucose was monitored which is considered as 0 min. Glucose (2 g/kg) was fed to each animal. Blood (0.1 ml) was withdrawn from the retro-orbital sinus under mild anesthesia at 30, 60 and 120 min after glucose load. Plasma was separated and glucose was estimated using biochemical analyzer.

Insulin tolerance test (ITT):

ITT was performed in overnight (12 h) fasted mice according to the method described by Thounaojam et al. (2010) [13]. The mice were allowed to drink water and the treatment groups were administered the respective drugs one hour before blood collection. Initial plasma glucose was monitored which is considered as 0 min. Insulin 0.75 U/kg was administered intraperitoneally to each animal 10 min after initial blood collection. Blood (0.1 ml) was withdrawn from the retro-orbital sinus under mild anesthesia at 30, 60 and 120 min after insulin administration. Plasma was separated and glucose was estimated using commercial available glucose estimation kit.

Exercise induction

Exercise was induced in the mice [14] belonging to the group 5 with the help of rota-rod apparatus (Inco, India) at 10 rpm for 15 min. Exercise was performed three times a week during 10.30 h to 11.30 h. At the end of the treatment week all the animals were made to perform exercise on the apparatus. Their muscle performance or endurance capacity was measured on the basis of their fall-off times from the rotating rod.

Estimation of SOD, MDA and Catalase in Skeletal muscle

The animals were sacrificed by cervical dislocation at the end of the treatment week. Gastrocnemius muscle isolated from both the hind limbs was homogenized and centrifuged [15]. SOD was estimated as per Marklund and Marklund [16]. Method of Aebi [17] was followed for Catalase estimation. MDA was estimated as described by Esterbauer and Cheeseman [18].

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Data were analyzed by using one-way analysis of variance (ANOVA) followed by dunnett's t-test as post hoc analysis. Statistical significance was determined at 5% level of confidence ($p<0.05$).

RESULTS AND DISCUSSION

Percentage yield of extract and standardization

The percentage yield of methanol extract of *Glycine max* was found to be 9.86%. The percentage yield of ethyl acetate extract after complexation was found to be 9.20% with crude methanol extract and 0.9% with soy seed powder. It was estimated by HPLC that isoflavones rich fraction of soya seed extract contains 16.22% w/w of daidzin.

Oral glucose tolerance test (OGTT)

OGTT was carried out at week 04. In diabetic control, plasma glucose level was significantly ($p<0.01$) high in both the phases. In all treatment groups, the slope of first phase was unchanged as compared to diabetic control mice. However, the plasma glucose was significantly ($p<0.01$) low as compared to diabetic control at 0 and 30 min. DRE treated and Exercise induced groups showed significant ($p<0.01$) lowering of glucose curve as compared to diabetic control. Group 4 and 6 showed significant ($p<0.01$) lowering of the slope in second phase indicating better glucose utilization. Impaired glucose tolerance is reflected in a larger incremental area under the curve (AUC) of the OGTT. The AUC of diabetic control was significantly ($p<0.01$) higher than non-diabetic group. The AUC was significantly ($p<0.01$) attenuated in groups 3, 4, 5 and 6 mice. Group 4 which is the combination of DRE and metformin showed a better glucose disposal as compared to other treated groups. The results are depicted in (Figure 1A and 1B).

Insulin tolerance test (ITT)

In diabetic control mice, plasma glucose was slowly reduced after 60 min of insulin injection. The plasma glucose level was normalized at 120 min. However, group 4 and 6 showed significant ($p<0.01$) glucose disposal in response to insulin. Group 4 and 6 showed marked improvement in glucose disposal. Group 4 found to be more effective than group 6. The plasma glucose level was significantly ($p<0.01$) reduced in group 3 and 5. However, the response in both group 3 and 5 was found to be equal. The result of the ITT is represented in (Figure 2).

Plasma glucose, Triglyceride and Total cholesterol level

The fasting plasma glucose level of non diabetic group was found to be normal at week 4. After high fat diet and MLDS (multiple low dose of STZ), plasma glucose level of diabetic control groups was profoundly increased upto 200 - 260mg/dl at 28 day. On prior treatment with DRE, plasma glucose level was significantly ($p<0.05$) reduced at week 04.Whereas, Met and DRE+ Met treated groups showed better reduction ($p<0.01$) in fasting plasma glucose level (Figure 3). Diabetic control mice showed significantly ($p<0.01$) increased in TC and TG in comparison to nondiabetic mice. On treatment with DRE, DRE + Met and Met, fasting plasma TC level was greatly ($p<0.01$) attenuated as compared to diabetic control at week 4 (Figure 3). All the groups demonstrated significant ($p<0.01$) reduction in plasma TG at week 04 in comparison to diabetic control. However, DRE + Met and Met treated groups showed better reduction in TG as compared to other groups (Figure 3).

Endurance capacity

On 28 day of experiment, the animals of all groups were induced exercise training and their endurance performance was estimated basing on their fall of time (in sec) from the rotating rod of the Rota-rod apparatus. The diabetic control spent little time (56 ± 3.1 sec) on the rotating rod in comparison to nondiabetic (99 ± 10.9 sec). Groups 3 and 4, showed significant ($p<0.01$) increase in the fall- off time with respect to the diabetic control. Group 5 mice were demonstrated maximum period of

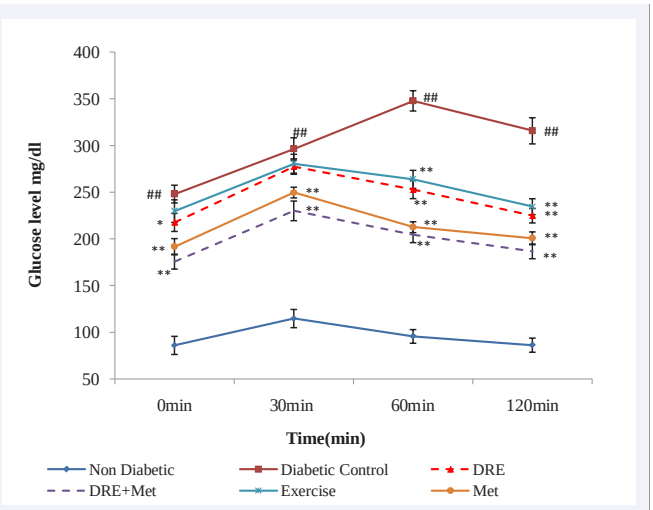

Figure 1a (A): Effect of standardized isoflavones rich fraction of soya seed extract on plasma glucose disappearance curve in OGTT at week 04. N=10; Values are expressed as mean ± SEM; DRE: Standardized isoflavones rich soya seed extract; Met: Metformin; ##p<0.01 vs. non diabetic group; *p<0.05 and **p<0.01 vs. diabetic control.

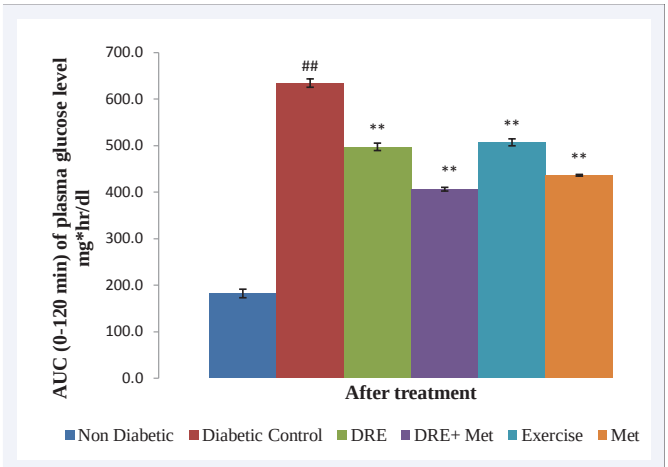

Figure 1b (B): Effect of standardized isoflavones rich fraction of soya seed extract on AUC of plasma glucose disappearance curve in OGTT at week 04.
N=10; Values are expressed as mean ± SEM; DRE: Standardized isoflavones rich soya seed extract; Met: Metformin; ##p<0.01 vs. non diabetic group; **p<0.01 vs. diabetic control.

muscle performance i.e. 240 ± 11.1 sec. Metformin treated mice showed significant ($p<0.05$) but minimal response (89 ± 4.9 sec) as compared to other treatment groups (Figure 4).

SOD, MDA and Catalase levels in skeletal muscle

Free radical scavenging activity and anti-oxidant status of DRE was monitored in skeletal muscle of mice. SOD level is significantly ($p<0.01$) lesser in diabetic control groups (7.8 ± 0.7 U/g of tissue) than non diabetic (11.1 ± 1.0 U/g of tissue). Whereas, DRE treated groups shows significant ($p<0.05$) improvement in their antioxidant level than Metformin. There was no significant increase in SOD activity in Met treated and exercise induced groups were observed. The SOD activity was significantly higher ($p<0.05$) in DRE (10.2 ± 0.9 U/g of tissue) and DRE+Met (10.7 ± 0.9 U/g of tissue) treated group (Figure

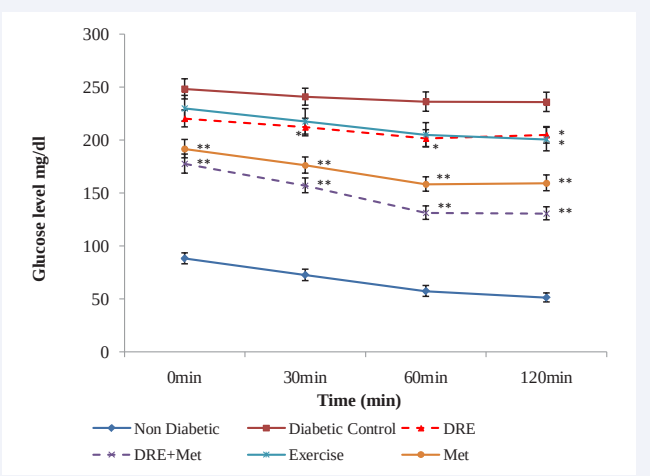

Figure 2 Effect of standardized isoflavones rich fraction of soya seed extract on plasma glucose level in ITT at week 04.
N=10; Values are expressed as mean ± SEM; DRE: Standardized isoflavones rich soya seed extract; Met: Metformin; ##p<0.01 vs. non diabetic group;*p<0.05 and **p<0.01 vs. diabetic control.

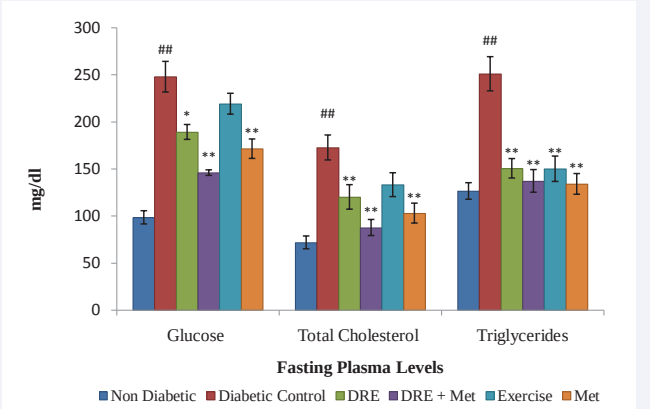

Figure 3 Effect of standardized isoflavones rich fraction of soya seed extract on fasting plasma glucose, total cholesterol and triglyceride level at week 04.
N=10; Values are expressed as mean ± SEM; DRE: Standardized isoflavones rich soya seed extract; Met: Metformin; ##p<0.01 vs. non diabetic group; *p<0.05 and **p<0.01 vs. diabetic control.

5). In diabetic control group, MDA (12.2 ± 0.9 nM/g of tissue) or lipid peroxidation activity was significantly (p<0.01) higher as compared to nondiabetic control (6.1 ± 0.4 nM/g of tissue). DRE considerable reduced this oxidative enzyme activity. DRE+ Met treated group showed significant (p<0.01) reduction in MDA level (7.9 ± 0.7 nM/g of tissue) in gastrocnemius muscle of mice. DRE, Met and exercise trained groups also showed significant (p<0.05) reduction in MDA level (Figure 5). Catalase, an antioxidant enzyme reduces the oxidative stress. It is significantly (p<0.01) lowered in diabetic control group (20.8 ± 0.6 U/g of tissue) than that of non diabetic animals (42.1 ± 4.1 U/g of tissue). Whereas, isoflavones (DRE and DRE+Met) treated groups showed significant increase in catalase activity (p<0.01) in skeletal muscle of mice. Exercise trained group has also significantly (p<0.01) increased the catalase activity in skeletal muscle of mice. The result is represented in Figure 5. DRE and DRE+Met combination showed better efficacy in MDA, SOD and catalase activity.

DISCUSSION

Type 2 diabetes (T2D) is a bipolar disease characterized by a defect in both insulin secretion and insulin action (insulin resistance) whose complex interaction leads to a progressive increase of plasma glucose levels. Various organs play a crucial role in the pathophysiology of T2D. Disruption of the cross-talk between pancreas, liver, skeletal muscle, adipose tissue and presumably, gut and central nervous system may lead to alteration of glucose homeostasis and T2D [19].

Insulin resistance is characterized by an overproduction of glucose by the liver and reduction of glucose utilization by skeletal muscle. T2D never occurs as long as pancreatic β-cells are able to compensate for insulin resistance by an over secretion of insulin (prediabetic state). The passage from prediabetic state to T2D is characterized by three major changes. The first one is a reduction

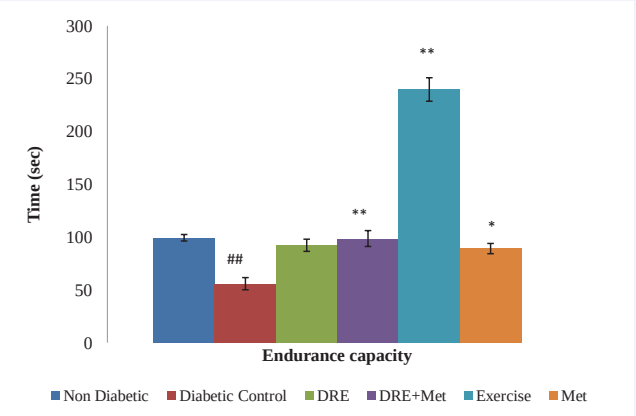

Figure 4 Effect of standardized isoflavones rich fraction of soya seed extract on endurance capacity using rota- rod apparatus at week 04.
N=10; Values are expressed as mean ± SEM; DRE: Standardized isoflavones rich soya seed extract; Met: Metformin; ##p<0.01 vs. non diabetic group; *p<0.05 and **p<0.01 vs. diabetic control.

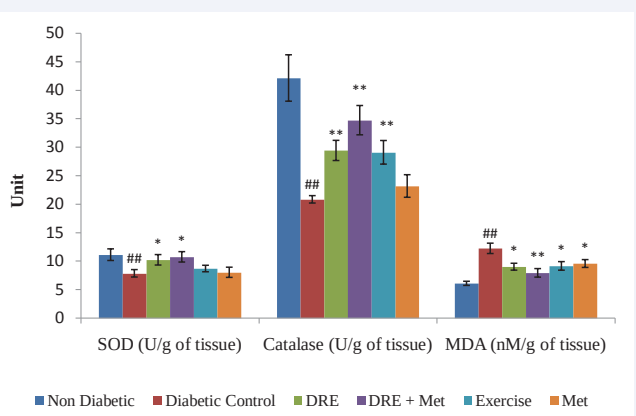

Figure 5 Effect of standardized isoflavones rich fraction of soya seed extract on SOD, MDA and catalase level of gastrocnemius muscle
N=10; Values are expressed as mean ± SEM; DRE: Standardized isoflavones rich soya seed extract; Met: Metformin; ##p<0.01 vs. non diabetic group; *p<0.05 and **p<0.01 vs. diabetic control.

of pancreatic β-cell and of compensatory insulin secretion. It is not known whether if this functional defect in β-cells is genetically programmed and/or acquired (glucotoxicity and/or lipotoxicity). The second one is an overproduction of glucose by the liver, probably secondarily to the over secretion of glucagon, to an excessive release of free fatty acids and adipocytokines by the adipose tissue. The third one is an increase of insulin resistance in skeletal muscles, frequently linked to the presence of obesity and an excessive release of free fatty acids and adipocytokines. In the present investigation, HFD and MLDS treatment in Swiss albino mice was used as a T2D model. This model is reported to have more close resemblance with the human T2D. Mice were fed high-energy diet for a period to induce mild insulin resistance at first, and then an injection of a low dose of STZ (40 mg/kg, i.p.) to make partial dysfunction of beta cell for suppressing the insulin secretion, which works as a compensation to insulin resistance with the result of persistent hyperglycemia. A low dose of STZ injection after one week feeding of the high-energy diet has shown a great effect to induce diabetes by markedly elevating serum glucose, TC and TG levels [10]. The results of the present experiment showed significant abnormalities in blood glucose, lipid profile, antioxidant status, food consumption, body weight, endurance capacity in diabetic control mice as compared to other treatment groups.

In addition to conventional therapeutic treatment, physical exercise is recommended for the prevention and management of T2D. It has been shown to attenuate diabetes induced energy metabolic changes in skeletal muscle. Diabetic muscles are more vulnerable than healthy muscles to exercise induced myofiber damage. The ability to increase muscle mass and rate of protein synthesis in skeletal muscle depends on the severity of the diabetes. Mitochondrial abnormalities have been associated with the progression of a variety of pathologies including diabetes. Insulin resistance emanating from mitochondrial dysfunction may contribute to metabolic abnormalities and subsequent increases in chances of diabetes. Several literatures suggest that insulin resistance is associated with decreased mitochondrial number, abnormal morphology, lower levels of mitochondrial oxidative enzymes and lower ATP synthesis in human muscle biopsies *[20].* Therefore, developing therapeutics to improve mitochondrial function and/or number is an attractive strategy for preventing cell death, preserving organ function and treating diabetes. PGC-1α has been characterized as a master regulator of mitochondrial biogenesis [21]. However, few pharmacological agents are known to increase mitochondrial biogenesis. It has been reported that daidzein, genistein, biochanin A, formononetin, increased expression of PGC-1α and resulted in mitochondrial biogenesis. In other reported path for mitochondrial biogenesis, activation and expression of SIRT1, a deacetylase and activator of PGC-1α is also influenced by isoflavone derivatives [22].

With this background information the present study was designed and studied for improvement in endurance capacity and glucose utilization in diabetic mice on administration of standardized isoflavones rich soya seed extract. Secondary outcomes like improved biochemical, antioxidant status was studied and compared with the exercise trained and metformin treated mice. HFD-MLDS induced diabetes in Swiss albino mice showed a very consistent model for diabetes throughout the study. It has been reported that fasting plasma glucose level in HFD-MLDS model, was more than 200 mg/dl after 28 days [10]. In our present investigation we found a consistent increase in fasting plasma glucose level in diabetic control. Plasma glucose level was > 250 mg/dl which is in agreement with the previously reported studies [10]. On treatment with DRE, DRE+Met and Met, plasma glucose was considerably attenuated. Metformin has been found to lower the plasma glucose by facilitating glucose utilization in skeletal muscle and reduced hepatic production of glucose [23-25]. Exercise increases mitochondrial fatty acid oxidation, which is believed to play an important role in maintaining muscle insulin sensitivity [26]. Thus, regular exercise could be beneficial for preventing and managing the blood glucose level in diabetic patients. However, Diabetic patients are highly intolerant to exercise and their endurance capacity decreases over the period of time. The benefit of soy isoflavones and soy protein has been proven in diabetic mice, in terms of lowered blood glucose level during OGTT [27]. Nevertheless, its effect on exercise endurance is not been studied.

OGTT is a simple test widely used in clinical practice to diagnose glucose intolerance and T2D. OGTT reflects the efficiency of the body to dispose of glucose after an oral glucose load or meal Impaired glucose tolerance is reflected in a larger incremental area under the curve (AUC) of the plasma glucose disappearance curve. Results of OGTT in this study revealed that the $AUC_{glucose}$ significantly increased in diabetic control in comparison to non-diabetic control. At the same time, it was noted a delayed absorption phase and slower disposal phase in diabetic control mice. However, Met and DRE+Met groups showed stiffer slope of absorption and also disposal phase. This confirms the faster glucose disposal in these groups. Further, it was observed that DRE+Met group showed better efficacy than Met treated group. DRE treatment along with metformin could have increased the insulin sensitivity in skeletal muscle and liver. Hence, DRE and Met treatment could have an additive effect. DRE treated group showed a mild but significant disposal phase which is almost at par with exercise induced group. We observed a blunted blood glucose utilization response during the ITT in our diabetic control group. This reflects lower insulin sensitivity in the diabetic control animals. Such response was also observed by Zhang et al. (2008) in HFD-MLDS diabetic rats [28]. Insulin dependent glucose disposal was observed to be significant in Met and DRE+Met treated group. This showed increasing insulin sensitivity in these groups. DRE and Met combination found to exhibits better response. Whereas, DRE alone displayed a minimal response and similar to exercise induced group. Both the tests i.e. OGTT and ITT established that DRE alone cannot improve the insulin sensitivity and glucose intolerance in HFD-MLDS diabetic mice. Although a minimal improvement was observed, it could be due to its effect on insulin secretion. Soya isoflavones have been reported to augment insulin secretion in STZ induced diabetic rats [5].

Increase in fasting plasma TG and TC were frequently shown in diabetes mellitus states [29,30]. In the present study, DRE and Met significantly attenuated the TG and TC level in plasma. Whereas, the fasting plasma TG and TC level was elevated greatly in diabetic control. It has been

Aerobic exercises develop slow twitch muscle fibers and thus help in enhancing the endurance capacity of an individual [31]. Rota-rod apparatus involves both balance and coordination as compared with simple locomotors exercise on treadmill. Hence motor balance and coordination training enhances functional outcome [14]. Previous investigations reported that spontaneous exercise activity or endurance capacity is decreased in STZ mice [32] and STZ-Nicotinamide rats [8]. We observed that endurance capacity was significantly reduced in our diabetic mice. Whereas, DRE treatment enhanced the exercise activity that is equivalent to non-diabetic mice. However, exercise induced mice showed highly significant improvement and stayed for longer period on the rotating rod. Exercise induced mice showed highly balanced and retaining capability due to their regular training. All other groups lack the balance capability on the rota-rod and fell-down early in the test. When we compare the fall-off period with diabetic mice, the values are significant for treatment groups. In our previous study, we observed similar results in STZ-Nicotinamide induced diabetic rats [8]. Kamiya et al., have reported that Isoflavones rich fraction of *Puerariae* flower increased the oxygen consumption and brown adipose tissue UCP1 expression in HFD fed mice [33]. Daidzin is a major isoflavonoid present in *Puerariae* flower and may be having increased mitochondrial activity. However, further investigations are required to substantiate the claim.

There are strong indications that oxidative stress may be a key event in diabetic complications [34,35]. Generation of ROS and production of AGEs is directly linked to chronic hyperglycemia [36-38]. Antioxidant enzymes and vitamins play major roles in eliminating lipid peroxides produced *in vivo* oxidative reactions. In the present experiments significant lower level of SOD and catalase was observed in skeletal muscle of diabetic control mice whereas, considerably increased level of MDA was observed. Upon treatment with DRE, Met and exercise induction, antioxidant enzyme levels were augmented and lipid per-oxidation was attenuated. The result of present study is in agreement with previously published results [39].

CONCLUSION

There are many effective anti-diabetic drugs available for therapeutic care. However, an agent which can improve the endurance capacity and metabolic flexibility can be an add-on therapy to the anti-diabetic agents. This can help the patient by reducing the anti-diabetic dose, possibility of hypoglycemia and other side effect caused by available drugs. Conclusively, our study demonstrated that isoflavones rich soya extract co-administered with metformin showed significant improvement in endurance capacity and glucose tolerance which is reflected in improved muscle performance, better glucose tolerance in OGTT and increased insulin responsiveness in ITT. On treatment with isoflavones, it has improved the blood parameters like fasting plasma glucose, total cholesterol and triglyceride. Further it has demonstrated a significant free radical scavenging activity with potential antioxidant effect that will benefit the diabetic patients to reduce the myopathy and improve the exercise performance. Overall results of the present investigation demonstrate isoflavones of soy extract could have two proposed mechanisms. First, it may increase the mitochondrial biogenesis and/or augmented mitochondrial activity in skeletal muscle without ROS production. Second, it has ability to increase the insulin sensitivity and antidiabetic effect on co-administration with metformin. However further studies on isoflavones should be designed with parameters like mitochondrial activity, mitochondria number, SIRT1 and PGC-1 α activity in skeletal muscle to validate the claim of increased endurance performance.

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REFERENCES

1. Risérus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. Prog Lipid Res. 2009; 48: 44-51.
2. King H, Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. Diabetes Care. 1993; 16: 157-177.
3. Friedenwald J, Ruhrah J. The use of the soy bean as a food in diabetes. Am J Med Sci. 1910; 140: 793–803.
4. Dang ZC, Audinot V, Papapoulos SE, Boutin JA, Löwik CW. Peroxisome proliferator-activated receptor gamma (PPARgamma) as a molecular target for the soy phytoestrogen genistein. J Biol Chem. 2003; 278: 962-967.
5. Lu MP, Wang R, Song X, Chibbar R, Wang X, Wu L, et al. Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. Nutr Res. 2008; 28: 464-471.
6. Rasbach KA, Schnellmann RG. Signaling of mitochondrial biogenesis following oxidant injury. J Biol Chem. 2007; 282: 2355-2362.
7. Rasbach KA, Schnellmann RG. Isoflavones promote mitochondrial biogenesis. J Pharmacol Exp Ther. 2008; 325: 536-543.
8. Bhattamisra SK, Mohapatra L, Panda BP, Parida S. Effect of isoflavone rich soya seed extract on glucose utilization and endurance capacity diabetic rat. Diabetol Croat. 2013; 42: 42–52.
9. Rao PR, Kumar KG, Narayanan MC. A new method of isolation of isoflavones from Glycine max by complexation technique. Asian J Sci Res. 2008; 1: 176–179.
10. Arulmozhi DK, Kurian R, Bodhankar SL, Veeranjaneyulu A. Metabolic effects of various antidiabetic and hypolipidaemic agents on a high-fat diet and multiple low-dose streptozocin (MLDS) mouse model of diabetes. J Pharm Pharmacol. 2008; 60: 1167-1173.
11. Richman JG, Kanemitsu-Parks M, Gaidarov I, Cameron JS, Griffin P, Zheng H, et al. Nicotinic acid receptor agonists differentially activate downstream effectors. J Biol Chem. 2007; 282: 18028–18036.
12. Bonner-Weir S. Morphological evidence for pancreatic polarity of beta-cell within islets of Langerhans. Diabetes. 1988; 37: 616-621.
13. Thounaojam MK, Jadeja RN, Devkar ARV, Ramachandran AV. Prevention of high fat diet induced insulin resistance in C57BL/6J mice by Sida rhomboidea Roxb. Extract. J Health Sci. 2010; 56: 92–98.
14. Ding Y, Li J, Lai Q, Azam S, Rafols JA, Diaz FG. Functional improvement after motor training is correlated with synaptic plasticity in rat thalamus. Neurol Res. 2002; 24: 829-836.
15. Oh-ishi S, Kizaki T, Ookawara T, Sakurai T, Izawa T, Nagata N, et al. Endurance training improves the resistance of rat diaphragm to

exercise-induced oxidative stress. Am J Respir Crit Care Med. 1997; 156: 1579-1585.

16. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47: 469-474.

17. Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105: 121-126.

18. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 1990; 186: 407-421.

19. Robertson RP. Oxidative stress and impaired insulin secretion in type 2 diabetes. Curr Opin Pharmacol. 2006; 6: 615-619.

20. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab. 2000; 279: 1039-1044.

21. Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, et al. Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. J Physiol. 2007; 583: 9-24.

22. Kuiper GG, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 1997; 138: 863-870.

23. Galuska D, Zierath J, Thorne A, Sonnenfeld T, Walberg- Henrikson H. Metformin increases insulin-stimulated glucose transport in insulin-resistant human skeletal muscle. Diabetes Metab. 1991; 17: 159–163.

24. Hundal HS, Ramlal T, Reyes R, Leiter LA, Klip A. Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. Endocrinology. 1992; 131: 1165-1173.

25. Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes. 2000; 49: 2063-2069.

26. Sherman WM, Costill DL, Fink WJ, Miller JM. Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. Int J Sports Med. 1981; 2: 114-118.

27. Lee DS, Lee SH. Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. FEBS Lett. 2001; 501: 84-86.

28. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2008; 2008: 704045.

29. Omoruyi FO, Grindley PB, Asemota HN, Morrison EYSA. Increased plasma and liver lipids in streptozotocin-induced diabetic rats: Effects of Yam (Dioscorea Cayenensis) or Dasheen (Colocassia Esculenta) extract supplements. Diabetol Croat. 2001; 30: 87–92.

30. Shim JY, Kim YJ, Lee HS. Effects of soybean isoflavone extract on the plasma lipid profiles and antioxidant enzyme activity in streptozotocin-induced diabetic rats. Nutr Res Pract. 2008; 2: 218-226.

31. Long YC. Skeletal muscle metabolic flexibility: the role of AMP-activated protein kinase and calcineurin. Karolinska Institutet, 2007.

32. Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Long-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol. 2005; 90: 827–835.

33. Kamiya T, Nagamine R, Sameshima-Kamiya M, Tsubata M, Ikeguchi M, Takagaki K. The isoflavone-rich fraction of the crude extract of the Puerariae flower increases oxygen consumption and BAT UCP1 expression in high-fat diet-fed mice. Glob J Health Sci. 2012; 4: 147-155.

34. Gorogawa Si, Kajimoto Y, Umayahara Y, Kaneto H, Watada H, Kuroda A, et al. Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in type 2 diabetes. Diabetes Res Clin Pract. 2002; 57: 1-10.

35. Kaneto H, Fujii J, Myint T, Miyazawa N, Islam KN, Kawasaki Y, et al. Reducing sugars trigger oxidative modification and apoptosis in pancreatic beta-cells by provoking oxidative stress through the glycation reaction. Biochem J. 1996; 320: 855–863.

36. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, et al. Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells. Diabetologia. 1999; 42: 1417-1424.

37. Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, Sbrana S, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes. 2005; 54: 727-735.

38. Reusch JE. Diabetes, microvascular complications, and cardiovascular complications: what is it about glucose? J Clin Invest. 2003; 112: 986-988.

39. Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life Sci. 2006; 79: 1578-1584.

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