

## Prevention of High Fructose-Induced Metabolic Syndrome in Male Wistar Rats by Aqueous Extract of *Tamarindus Indica* Seed

Mohammad Reza Shahraki<sup>1</sup>, Mehdi Harati<sup>2</sup>, and Ahmad Reza Shahraki<sup>3</sup>

<sup>1</sup> Department of Physiology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>2</sup> Departments of Biochemistry, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>3</sup> Faculty of Medicine, Zahedan University of Medical Sciences, Member of Young Researchers Club of Zahedan Azad University, Zahedan, Iran

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**Abstract-** *Tamarindus indica* is used as a traditional treatment for diabetes. To elucidate whether *Tamarindus indica* seed aqueous extract (TSE) ameliorates metabolic syndrome in hyperinsulinemic rats, we evaluated serum insulin, dehydroepiandrosterone sulfate (DHEAS), triglyceride (TG), total cholesterol (TC), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and glucose levels in fructose-fed rats. Animals were divided into three groups; control (C) receiving tap water, fructose-fed (F) and TSE-treated fructose-fed rats (F-T) both receiving tap water supplemented with 10% (w/v) fructose. Water was prepared every day for a period of 8 weeks for all three groups. F-T rats were fed with TSE via gavage feeding at the dose of 20 mg/0.5 ml distilled water/100 g body weight per day. Fasting serum glucose levels of three groups were comparable. TSE treatment prevented the increase in fasting serum insulin, TG, TC, VLDL, and LDL in the F-T group ( $P<0.01$ ) when comparing with the F group. Fructose feeding led to a decrease in fasting serum DHEAS, and HDL levels in the F group ( $P<0.01$ ) compared with the control. TSE treatment prevented the decrease in fasting serum DHEAS, and HDL levels in the F-T group ( $P<0.01$ ) while these results were not seen in control rats. It is indicated that the hyperinsulinemia in fructose-fed insulin resistant rats are associated with low levels of DHEAS, and HDL; and high levels of TC, VLDL, LDL, and TG. TSE supplementation probably ameliorates metabolic syndrome due to the improved insulin action.

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**Keywords:** *Tamarindus indica*; Metabolic syndrome X; Fructose; Dihydroepitesterone; Dyslipidemias; Rats

### Introduction

Epidemiological studies have clearly demonstrated that atherosclerosis stems from several accumulating clinical conditions such as dyslipidemia, hyperinsulinemia with insulin resistance, glucose intolerance, and hypertension at the same time (1). This state - referred to as metabolic syndrome, also referred to as insulin resistance or syndrome X – proves that the increasing incidence of diabetes in combination with obesity is a result of changes in human behavior, available nutrition, and the adoption of more sedentary lifestyles (2). Metabolic syndrome is frequently associated with lipoprotein abnormalities namely hypotriglyceridemia, high levels of VLDL and LDL; and low levels of HDL which serve

as risk factors for atherosclerosis (3). In addition, previous studies have indicated that insulin resistance could induce a decrease in dehydroepiandrosterone (DHEA) levels and its sulfate ester (DHEAS) in human (4,5). DHEA and DHEAS are the most abundant circulating adrenal steroids in human (6). Apart from their role as precursors of androgens and estrogens, their precise biological functions are still unknown (7). Circulating levels of DHEA and its sulfate ester decline with age and a relationship has been suggested between lowered DHEA levels and heart disease, cancer, diabetes, obesity, chronic fatigue syndrome, AIDS and Alzheimer's disease (8,9). In spite of the fact that antidiabetic agents have been widely introduced, diabetes and its related complications continue to be a

**Corresponding Author:** Mohammad Reza Shahraki

Department of Physiology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran  
Tel: +98 541 2419403, 915 3415608, Fax: +98 541 2442481, E-mail: M\_shahrakim@ZAUMS.ac.ir

major medicinal problem; therefore, new agents capable of augmenting insulin sensitivity at muscle and liver levels, may be useful in the management of insulin resistance in type 2 diabetes mellitus patients. In recent years, there has been a renewed interest in plant medicine for the treatment of lots of disease such as diabetes (10). Furthermore, evidence has indicated that some special plant species exert multiple antidiabetic effects (11-13).

*Tamarindus indica* has been traditionally used for the management of diabetes mellitus in human and experimental animals (14-16). *Tamarindus indica* is a plant belonging to the *Caesalpiniaceae* family and it is mostly found all over India and other tropical countries. The plant fruits mainly in summer. The seeds are dicotyledonous and brownish black in color, though the kernels are white. Previous reports have shown that fructose-enriched diet developed insulin resistance and dyslipidemia in human and animal models (17,18). This diet caused metabolic effects similar to those observed in syndrome X in which insulin resistance, hypertension and dyslipidemia are observed among patients with metabolic syndrome (19). The attenuating effect of *Tamarindus indica* seed aqueous extract on fructose-fed hyperinsulinemic rats has not been previously investigated. In the light of our preliminary study, in which we indicated that insulin resistance induces dyslipidemia and decreases serum DHEAS (20), we have undertaken to evaluate the preventive effects of aqueous extract of *Tamarindus indica* seed on high-fructose induced metabolic syndrome in male Wistar rats.

## Patients and Methods

### Chemicals

Fructose was purchased from Merck Chemicals, Germany and all other chemicals used in this study were of analytical grade.

### Plant material

Seeds of *Tamarindus indica* were obtained from a commercial source.

### Preparation of aqueous extract of *Tamarindus indica* seed

Seeds of *Tamarindus indica* were dried in an incubator for 2 days at 40 °C, crushed in an electrical grinder, then powdered. Extraction was performed by mixing 25 g of powder in 250 ml of distilled water in a soxhlet apparatus for 18 hours. The product was a dark

brown aqueous extract which later was dried in incubator for 1 day at 40°C.

### Animal study

The study was carried out using twenty-nine matured normoglycemic male Wistar rats, weighing  $140 \pm 10$  g, which were separately housed in cages (one rat per cage) and had free access to water and food sources. Animals were maintained in a room at  $23 \pm 2^\circ\text{C}$  with a fixed 12-h artificial light period and the air was adequately recycled. All animals were fed with standard rodent diet.

### Induction of metabolic syndrome

Fructose-induced metabolic syndrome was induced in normoglycemic male Wistar rats by adding fructose solution 10% (w/v) in tap water that was prepared every day.

### Experimental design

Twenty-nine rats were randomly divided into three groups as follows:

- (i) Control (C): Rats in this group (n=9) received standard rodent diet and tap water.
- (ii) Fructose-fed (F): Rats in this group (n=10) received standard rodent diet and tap water supplemented with 10% (w/v) fructose.
- (iii) TSE-treated fructose-fed (F-T): Rats in this group (n=10) received standard rodent diet and tap water supplemented with 10% (w/v) fructose, and *Tamarindus indica* seed aqueous extract administered at the dose of 20 mg/0.5 ml distilled water/100 g body weight per day by gavage.

Food and fluid intake of all above rat groups were measured daily and body weight were measured every two weeks.

The experiment was carried out for 8 weeks. At the end of the treatment period and after an overnight fast, all animals were sacrificed under light ether anesthesia. Blood samples were collected from tail vein. Blood serum was removed and stored at  $-20^\circ\text{C}$  for further analyses.

### Assay

Serum AST (Aspartate Aminotransfrase), ALT (Alanine Aminotransferase), glucose, TG, and TC levels were measured by standard methods adapted for a RA 1000 analyzer (Technicon, USA). Serum HDL was measured by precipitation of non-HDL lipoproteins with dextran/MgSO<sub>4</sub> followed by enzymatic cholesterol assay. LDL calculated using Friedewald formula (21). The formula hinges on the assumption that VLDL is present in a concentration equal to one fifth of TG's.

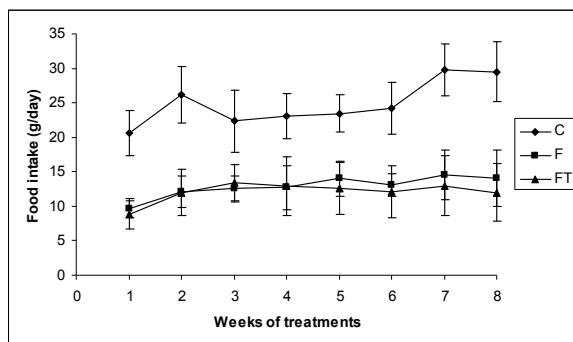
Serum insulin levels were determined by ultra sensitive rat insulin kit (DRG, France), using double-antibody enzyme-linked immunosorbent assay (ELISA). Serum DHEAS levels were determined by radioimmunoassay, using commercial kit (Immunotech, France).

### Statistical data analysis

Results were reported as mean  $\pm$  SE. To confirm the normal distribution, the data were analyzed by one-sample Kolmogorov-Smirnov test and then by Levene's test. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons were employed to compare differences among experimental groups. Significance level was set at  $P < 0.05$ . All statistical analyses were performed using SPSS 11 for Windows software system.

### Results

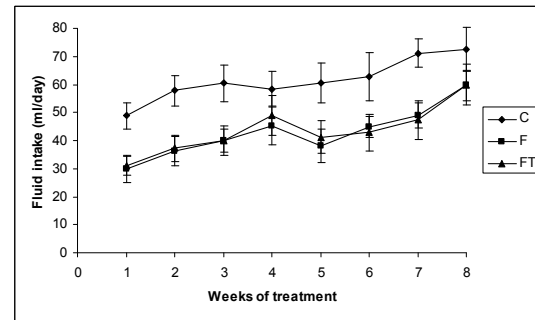
The effects of fructose feeding and TSE on food and fluid intake; body weight in different groups for period of 8 weeks have been respectively shown in figures 1, 2, and 3. As shown in figures 1 and 2; food and fluid intake of the F and F-T groups did not change at any time point. In contrast, food and fluid intake were significantly decreased in the F and F-T groups when compared with the control group ( $P < 0.01$ ). The body weight of C and F rats were similar. The body weight of F-T rats significantly decreased ( $P < 0.01$ ) compared with those of C and F groups (Figure 3).



**Figure 1.** Effects of fructose feeding and aqueous extract of *Tamarindus indica* seed on food intake in experimental groups for the period of 8 weeks. Values are mean  $\pm$  S.E.M

C = Control rats. F = fructose-fed rats, F-T = fructose-fed TSE treated rats.

No significant difference was noted between F and F-T groups at any time point. Significant differences were noted among F, F-T and control groups at any time point ( $P < 0.01$ ).



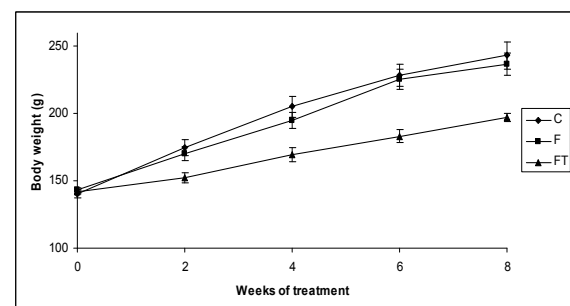
**Figure 2.** Effects of fructose feeding and aqueous extract of *Tamarindus indica* seed on fluid intake in experimental groups for the period of 8 weeks. Values are mean  $\pm$  S.E.M.

C = Control rats. F = fructose-fed rats, F-T = fructose-fed TSE treated rats.

No significant difference was noted between F and F-T groups at any time point. Significant differences were noted among F, F-T and control groups at any time point ( $P < 0.01$ ).

The effects of fructose feeding and TSE on serum glucose, TG, TC, LDL, VLDL, HDL, insulin, DHEAS, and fasting insulin/fasting glucose as an insulin resistance index are shown in tables 1 and 2. As shown in table 1, fasting serum glucose concentrations of the three groups were comparable at the end of the treatment period.

In contrast, fasting serum levels of TG, TC, LDL were significantly increased in F rats when compared with control ( $P < 0.01$ ). Fasting serum levels of TG, TC, LDL were significantly decreased in F-T rats compared with the F group ( $P < 0.01$ ). Moreover, fasting serum levels of HDL significantly decreased in the F group when compared with control rats ( $P < 0.01$ ), but fasting serum levels of HDL increased in F-T rats in comparison with the F group ( $P < 0.01$ ).



**Figure 3.** Effects of fructose feeding and aqueous extract of *Tamarindus indica* seed on body weight in experimental groups for the period of 8 weeks. Values are mean  $\pm$  S.E.M

C = Control rats. F = fructose-fed rats, F-T = fructose-fed TSE treated rats.

No significant difference was noted between F and control groups at any time point. Significant differences were noted among F-T, F and control groups at any time point ( $P < 0.01$ ).

**Table 1.** Fasting serum glucose, TG, TC, LDL, and HDL levels in experimental rats (after 8 weeks).

|                  | C (n=9)      | F (n=10)       | F-T (n=10)     |
|------------------|--------------|----------------|----------------|
| Glucose (mmol/L) | 6.22 ± 0.83  | 6.43 ± 0.90 *  | 5.75 ± 0.85 *  |
| TG (mmol/L)      | 1.08 ± 0.08  | 2.12 ± 0.11 ** | 1.25 ± 0.09    |
| TC (mmol/L)      | 1.97 ± 0.07  | 2.63 ± 0.11 ** | 2.02 ± 0.09    |
| LDL (mmol/L)     | 0.52 ± 0.05  | 0.98 ± 0.13 ** | 0.64 ± 0.05    |
| VLDL (mmol/L)    | 0.49 ± 0.04  | 0.97 ± 0.05 ** | 0.57 ± 0.03    |
| HDL (mmol/L)     | 0.95 ± 0.06  | 0.67 ± 0.03 ** | 0.91 ± 0.05    |
| AST (U/L)        | 65.7 ± 7.32  | 71.2 ± 6.56 *  | 77.48 ± 8.44 * |
| ALT (U/L)        | 34.45 ± 5.76 | 39.27 ± 7.63 * | 41.37 ± 9.55 * |

Values are means ± S.E.M. number of animals in each group is indicated in parenthesis. Comparisons were made by one-way ANOVA test.

C = Control rats. F = fructose-fed rats, F-T = fructose-fed TSE treated rats.

TG = triglyceride, TC = Total cholesterol, LDL = Low density lipoprotein, HDL = High density lipoprotein, VLDL = Very low density lipoprotein.

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase.

\* NS, compared to control rats.

\*\* ( $P < 0.01$ ), compared to control rats.

‡ ( $P < 0.01$ ), compared to F rats.

As shown in table 2, F rats were significantly hyperinsulinemic compared with the control group ( $P < 0.01$ ). Fasting serum insulin level significantly decreased in the F-T group compared with the F group ( $P < 0.01$ ). In other words, in the F-T group, the serum insulin level reached those of normoinsulinemic control rats. Fructose-enriched diet also led to a decrease in serum DHEAS concentration in the F group compared with control animals ( $P < 0.01$ ), but TSE treatment prevented this decrease in serum DHEAS concentration in the F-T group compared with the F group ( $P < 0.01$ ). The F rats also had higher insulin resistance index (fasting insulin/ fasting glucose) than control rats ( $P < 0.01$ ). In addition, TSE decreased insulin resistance index significantly in the F-T group compared with F animals ( $P < 0.01$ ).

AST and ALT serum activity were measured to assess liver function. As can be observed in Table 1, no significant alterations in liver aminotransferase activity are detected in the animal groups treated with the high fructose diet and TSE during 8 weeks when compared with the control group (Table 1).

## Discussion

The present study showed that treatment of fructose-fed hyperinsulinemic rats with TSE for the period of 8 weeks significantly reduced serum TG, TC, VLDL, LDL, insulin levels, insulin resistance index and increased serum HDL and DHEAS levels.

A well-established epidemiological link exists between hyperinsulinemia and macrovascular disease. Recent evidences have indicated that DHEA and DHEAS exert multiple antiatherogenic effects (22-25). Furthermore, recent studies in human have shown that elevation of serum insulin level to its high physiological range inhibits adrenal production of DHEA. This is achieved by selective suppression of 17, 20-lyase steroidogenic enzyme activity (26,27). It has been hypothesized that hyperinsulinemia promotes macrovascular disease in part by reducing plasma DHEA and DHEAS levels. It, additionally, illustrates how this may be the case in two clinical conditions characterized by hyperinsulinemic insulin resistance: aging and obesity (26).

**Table 2.** Fasting serum insulin, DHEAS levels, and insulin resistance index in experimental rats (after 8 weeks)

|                                  | C (n=9)      | F (n=10)       | F-T (n=10)   |
|----------------------------------|--------------|----------------|--------------|
| Insulin (pmol/L)                 | 79.32 ± 3.21 | 164.32 ± 6.69* | 76.25 ± 5.27 |
| DHEAS (μmol/L)                   | 0.65 ± 0.08  | 0.34 ± 0.02*   | 0.74 ± 0.06  |
| Fasting insulin/ fasting glucose | 12.75 ± 3.86 | 46.95 ± 7.43*  | 14.52 ± 8.74 |

Values are means ± S.E.M. Number of animals in each group is indicated in parenthesis. Comparisons were made by one-way ANOVA test.

DHEAS = Dehydroepiandrosterone sulfate.

\* ( $P < 0.01$ ), compared with control rats.

( $P < 0.01$ ), compared with F rats.

Moreover, elevated levels of plasma LDL, TG, accompanied by reduced HDL levels, are associated with an increased risk of coronary heart disease (3,28,29). However, the exact mechanism by which hyperinsulinemia promotes atherosclerosis is yet unclear.

Results of this study also showed that fructose-enriched diet could induce dyslipidemia in fasting rats. Previous studies in rats and other rodents (e.g.: hamster) also showed the same results (30, 31). Several reasons for insulin resistance in high fructose-fed rats have been reported. This phenomenon is believed to be related to the hypertriglyceridemic effect of fructose (32). Fructose feeding stimulates the hepatic production of triglycerides, both by promoting the reesterification of circulating nonesterified fatty acids and by stimulating *de novo* fatty acid synthesis (31). Increased delivery of triglycerides or nonesterified fatty acids to the muscle interferes with the utilization of glucose, through the principles of Randle cycle (33), and impairs insulin action. More recently, studies have identified an interesting link between the development of insulin resistance and deregulation of intestinal lipoprotein metabolism (34). Chronic fructose feeding stimulates the secretion of apolipoproteins mostly in the form of B48-containing lipoprotein particles accompanied by enhancing intestinal lipid synthesis especially free cholesterol, cholesterol ester, and triglyceride, as well as increasing both microsomal triglyceride transfer protein mass (MTP) and activity (35). These results suggest that in insulin-resistant or diabetic animals, there may be a mechanism responsible for enhanced intestinal secretion of lipoproteins in the fasted state. Fructose feeding may enhance the basal levels of lipoprotein secretion through increased *de novo* lipogenesis and increased MTP availability, as well (35).

Fructose-fed animals showed a significant shift toward secretion of the large, less dense, triglyceride-rich lipoprotein i.e. VLDL in the insulin resistance state, (36) and other lipoproteins and lipids such as LDL (37). Hence, our results are supported by previous reports. Reports, additionally, indicate that aqueous extract of *Tamarindus indica* seed attenuates hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats (14,15), and *Tamarindus indica* pulp fruit extract exhibits hypolipemic effects in hypercholesterolemic hamsters (16). Our data, for the first time, demonstrated that TSE ameliorates hyperinsulinemia in fructose-fed insulin resistant rats. On the other hand, it is known that high levels of blood-circulating triglyceride interfere with insulin function through its receptors (32).

Therefore, data from this study indicates that improvement of insulin physiological activity and prevention of insulin resistance are achieved by TSE may be, at least in part, due to an increase in insulin receptor binding.

As can be observed, no significant difference in food and fluid consumption was noted between the F and F-T groups at any time point. In contrast, significant reduction was notable in F and F-T rats in comparison with the control group. Furthermore, no significant difference in body weight was observed between the F and control groups at any time point while significant reduction was seen in the F-T group compared with F and control rats. In other words, although fructose solution (10% w/v) reduced food intake (probably by producing high amounts of calorie and energy) and water intake (probably because of its sweet taste), supplementation with TSE had no significant effect on food and water consumption. Thus, no reduction of appetite was accompanied by TSE-induced weight loss in F-T rats. This hypolipemic effect may have its origins in elevation of serum HDL which transfers cholesterol from tissue to liver for metabolism. Also, TG-lowering effect of TSE may contribute to inhibition of VLDL secretion by liver and increase VLDL clearance via lipoprotein lipase pathway. Further studies need to demonstrate mechanism of action of TSE.

Over and above ruling out a possible damage to the liver by TSE, we evaluated the serum aminotransferase activity. Treatment with this extract did not present any significant alternation in serum AST and ALT activity.

In conclusion, the present work provided additional evidence on previous assumptions that feeding with high doses of fructose lead to hyperinsulinemia along with reduction of DHEAS and non-HDL hyperlipidemia, potentially important side effects of diabetes. It is most likely that improved insulin action by means of aqueous extract of *Tamarindus indica* seed could be responsible for the amelioration of metabolic syndrome side effects. Finally, this extract may be useful in overcoming diabetes mellitus complications such as atherosclerosis as it has already been shown to associate with low levels of serum DHEAS and HDL; and high levels of non-HDL lipoprotein. Further investigation is required on the beneficial effects of treatment with aqueous extract of *Tamarindus indica* seed in diabetic patients.

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