Antihyperglycemic Activity and Body weight effects of Extracts of Emblica officianalis, Tamarix nilotica and Cinnamon Plant in Diabetic Male Rats

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Abstract

The study investigated the anti-hyperglycemic potential of aqueous extracts of *Emblica officinalis* fruit, *Tamarix nilotica* leaf and cinnamon sticks for six weeks on streptozotocin induced (70 mg/kg) diabetic obese rats compared to metformin as a standard drug. Six groups were administered plant extracts at two doses (200 mg/kg b.wt, 400mg/kg b.wt) with two groups as the positive and negative controls. Blood glucose, body weights, cholesterol, triglycerides, and creatinine levels were assessed. All plant extracts doses; significantly ($p\leq0.001$) reduced blood glucose levels among diabetic rats in the fifth and sixth weeks except the group treated with the lower dose of cinnamon. Body weights significantly increased during the fourth, fifth and sixth weeks, being more pronounced in the plant treated diabetic groups compared to the diabetic control and metformin group. Furthermore, all doses showed significant decrease ($p\geq0.05$) in serum glucose, cholesterol, triglyceride, and levels compared to the metformin, however, there was no significant serum creatinine effect. The anti-diabetic potential of the plant extracts used was dose-independent and there was no significant difference observed within the plant treated groups. However, all the plant extract used proved better than metformin. This suggests a marked anti-diabetic potential of the plant extracts attributed to their polyphenolic constituents.

Keywords: Phyto-compounds, diabetes, obesity, metformin
1. Introduction

The global epidemic of type II diabetes mellitus (DMT2) and obesity are on the rise and at the current rate the estimates for the year 2000 through 2030 show an increase from 171-366 million patients [1]. The pattern of prevalence has been the same in the Gulf including Saudi Arabia. Saudi Arabia has experienced an exponential socioeconomic growth over the past few decades which have led to a sedentary and affluent lifestyle of the people in the urban society. A recent follow up epidemiological study shows an alarming increase in the prevalence of DMT2 during the past few years [2]. This has stimulated public awareness of this endocrine disorder and the identification of risk factors associated with it.

The causes of type 2 diabetes and obesity are multifactorial among which, diet definitely plays a role in the incidence and severity of these syndromes. Dietary components beneficial in the prevention and treatment of these diseases have not been clearly defined, but it is postulated that phytoneutrients may play a role and believed to be less toxic and free of side effects than the synthetic drugs commonly used in therapy. Spices such as cinnamon, turmeric, amla fruit, and *Tamarix nilotica* leaf display insulin-enhancing activity [3]. Botanical products can improve glucose metabolism and the overall condition of diabetic individuals, not only by improving lipid metabolism, antioxidant status, and capillary function [4].

The fruits of *Emblica officinalis* Gaertn commonly known as amla or Indian gooseberry is known for its medicinal and therapeutic properties from ancient time in India and considered as a wonder fruit for health conscious population. It is extensively found throughout India and some other Asian countries. The fruits are widely consumed raw, cooked, or pickled and are widely used for their preventive, curative, and health restorative properties [5]. *Tamarix nilotica* (Ehrenb) has its root deep in the Egyptian history where it was mentioned in ancient papyri in pharaonic times to expel fever, relieve headache, to draw out inflammation and as an aphrodisiac, in addition, it was used in Egyptian traditional medicine as an antiseptic agent [6]. Cinnamon supplementation is known to facilitate glucose disposal in healthy humans, which may be achieved by enhancing (a) insulin sensitivity via increased phosphorylation of signalling proteins and (b) insulin sensitive glucose transporter 4-mediated glucose uptake into muscle cells. Because insulin also plays a key role in lipid metabolism, the present study postulated that consumption of herbal extracts would lead to improved glucose and blood lipids.

Therefore, this study was designed to determine whether there is a dose response of *Emblica officinalis*, *Tamarix nilotica*, and cinnamon on male diabetic rats. The authors assessed blood glucose, body weights and other related markers as serum cholesterol, triglycerides and creatinine associated with the progression of diabetes, in streptozotocin (STZ) induced diabetic rats. A comparative assessment of the plant treatments was also made with metformin, a commonly used antidiabetic drug.

2. Materials and Methods

2.1 Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade. Streptozotocin was used to induce diabetes (Sigma, U.S.A.). Metformin used to control diabetes (Merck Serono Middle East). Commercial kits for the estimation of serum glucose, cholesterol, triglycerides and creatinine (United Diagnostic Company, Saudi Arabia).
2.2 Plant material and Preparation of extract
Air dried fruits of *Emblica officinalis* and cinnamon sticks were procured from an Indian grocery store in Riyadh City, Saudi Arabia. Fresh leaves of *Tamarix nilotica* were purchased from a grocery store in Riyadh City, Saudi Arabia. The leaves were identified by the Department of Botany at King Saud University, Riyadh.

Preparation of aqueous amla (*Emblica officinalis*) fruit extract: Shade dried amla fruit were obtained from the local market in Riyadh. 100 gm of dried amla fruits were ground in an electrical grinder and dissolved in 500ml distilled water. The mixture was left for 24 hrs with a magnetic stirrer at room temperature. The next day the mixture was strained out in a fine sieve and the crude extract was air evaporated for 3 days. The concentrated fruit extract was then orally administered to the rats in different treatment groups (200 mg/kg and 400 mg/kg body weight) using a syringe.

Preparation of aqueous *Tamarix niloticus* leaf extract: For the leaf extract, 100 gm of shade dried leaves were ground in an electrical grinder and dissolved in 500 ml distilled water. The mixture was left for 24 hrs with a magnetic stirrer at room temperature. The next day the mixture was strained out in a fine sieve and the crude extract was air evaporated for 3 days. The concentrated leaf extract of each plant was then orally administered to the rats in the different treatment groups (200 mg/kg and 400 mg/kg body weight) using a syringe. The same procedure was followed with the cinnamon sticks.

2.3 Experimental animals
Male Wistar rats weighing 200-250 gm were used. The animals were fed with standard laboratory chow and had free access to water under well ventilated conditions of 12 hrs day and 12 hrs dark cycles. The animals were acclimatized to laboratory conditions prior to the experiment. The protocols were approved by the National Committee for Medical and Bio-ethics at our institute. The animals were handled according to standard protocols for the use of laboratory animals.

2.4 Induction of diabetes
The rats were made to fast 12 hrs before the induction of diabetes. Thereafter, they were injected with streptozotocin (70 mg/kg, i.p.). Five days after injection the rats with fasting blood glucose higher than 200 mg/dl were considered diabetic and used for the experiment. Feeding was stopped 12 hrs before blood sampling.

2.5 Experimental design
The experimental period was six weeks. The first 5 days were for the induction of diabetes in rats and the rest was the investigational period with crude aqueous extracts of *Emblica officinalis* fruit, *Tamarix nilotica* leaf, and cinnamon sticks which were administered separately. There were nine groups of five rats each.

Group-1: Normal saline treated rats (Normal control-NC)
Group-2: Normal saline treated diabetic rats (Diabetic Control-DC)
Group-3: Metformin (600 mg/kg body weight) treated diabetic rats (MF)
Group-4: *Emblica officinalis* aqueous fruit extract (200 mg/kg body weight) treated diabetic rats (A1-4 ml)
Group-5: *Emblica officinalis* aqueous fruit extract (400 mg/kg body weight) treated diabetic rats (A2-8 ml)
Group-6: *Tamarix nilotica* leaf aqueous extract (200 mg/kg body weight) treated diabetic rats (B1-4 ml)
Group-7: *Tamarix nilotica* leaf aqueous extract (400 mg/kg body weight) treated diabetic rats (B2-8 ml)
Group-8: Cinnamon sticks aqueous extract (200 mg/kg body weight) treated diabetic rats (C1-4 ml)
Group-9: Cinnamon sticks aqueous extract (400 mg/kg body weight) treated diabetic rats (B2-8 ml)

2.6 Blood sampling and biochemical analysis
Before and after administration of the aqueous plant extracts and metformin, rats were anaesthetized using carbon dioxide. Venous retro orbital blood samples [9] were collected in the fasting state at specific intervals using a glass capillary and collected in polystyrene tubes without the anticoagulant. Serum was separated by centrifugation at 3000 rpm for 10 mins after which it was tested for glucose. At the end of the experimental period, the blood samples collected were also tested for serum cholesterol, triglycerides and creatinine. Samples were stored at -20°C until assayed.

2.7 Statistical Analysis
Results were presented as the mean standard deviation (SD). A one-way analysis variance (ANOVA) was performed using SPSS-17 statistical software. Paired t-test was used for multiple comparisons. The values were considered significantly different when the p-value was lower than 0.05.

3. Results

3.1. Antihyperglycemic studies in STZ diabetic rats (70 mg/kg)
There was no mortality during the experimental period. The basal serum glucose levels of the rats from all the experimental groups were comparable (105-109 mg/dl) and there was no significant difference observed between the groups. In the first week, after the administration of STZ (70 mg/kg), the blood glucose level was increased in all the diabetic groups, the increase being almost four-fold (362-432 mg/dl). Within all diabetic groups (DC, A1, A2, B1, B2, C1, C2 and MF), the blood glucose level was comparable but significantly (p≤0.05) higher than the normal control (NC).

During weeks 2, 3 and 4 there was no significant change observed in the blood glucose level of the rats from all treated groups (A1, A2, B1, B2, C1, C3 and M) in comparison to the diabetic control (DC) group. However, in week 5, the blood glucose level of rats from all treated groups showed a highly significant (p≤0.001) decrease in comparison to the diabetic control, though there was no significant difference observed within the treated groups. During the last week (week 6) of the experimental period, the blood glucose level was further reduced in all treated groups being highly significant (p≤0.001) from the diabetic control. The blood glucose level of the groups treated with the plant extracts, A1, A2, B1, B2, C2 was also significantly (p≤0.001) lower than the group treated with metformin (MF) except the group treated with the lower dose of cinnamon extract, C1 (figure 1).
Overall, the plant extract treatments at both low and high doses used in the present study, *Emblica officianalis* fruit extract (A1, A2), *Tamarix nilotica* leaf extract (B1, B2) and Cinnamon extract (C1, C2) lowered the blood glucose level in the diabetic rats in the fifth and sixth week of the experimental period. The antihyperglycemic effect of plant extracts used was significantly better than the anti-diabetic drug, metformin, specifically in the sixth week of the experiment. However, there was no significant difference observed within the plant treated groups and between the low and high doses of the plant extracts used.

![Graph of blood glucose levels over time for different groups](image)

**Figure 1.** Effect of aqueous extracts of *Emblica officianalis* fruit, *Tamarix nilotica* leaf and Cinnamon on serum blood glucose level (mg/dL) of diabetic rats from Week 1 to Week 6. Groups: NC-Normal Control, DC- Diabetic Control, MF- Metformin treated, A1-(*Emblica officianalis* fruit extract, 200mg/kg), A2-(*Emblica officianalis* fruit extract, 400 mg/kg), B1-(*Tamarix nilotica* leaf extract, 200mg/kg), 2-(*Tamarix nilotica* leaf extract, 400 mg/kg), C1-(Cinnamon extract, 200mg/kg), C2-(Cinnamon extract, 400mg/kg).

### 3.2. Body weight

The mean basal body weight of all experimental groups ranged from 160 gm -177 gm and there was no inter-group variation observed. Post administration of STZ, in week 1, the body weight increased in all experimental groups but was statistically insignificant. The same pattern was observed in weeks 2 and 3. However, a significant (p ≤ 0.01) increase in the body weight was observed in all experimental groups in weeks 4, 5 and 6. The body weight of diabetic controls was significantly (p ≤ 0.05) less in comparison to the plant treated groups, A1 (week 4), A2 (week 6), and B2 (weeks 4 and 5). The body weight of the plant treated groups, A1, B2 (p ≤ 0.01), C1, C2 (p ≤ 0.05) was also significantly higher than the metformin treated group, MF in week 4. Further, in week 5, groups treated with *Tamarix* leaf extract, B1 (p ≤ 0.05) and B2 (p ≤ 0.01) showed a significantly higher body weight than the metformin treated group. In week 6, the MF group recorded a body weight significantly (p ≤ 0.05) less than the group treated with Amla extract, A1 and A2. There was no significant difference observed in the body weight within all
the plant extract treated groups. The body weight of the normal control group was also statistically non-significant to all the diabetic groups including the diabetic control (figure 2).

Figure 2. Effect of aqueous extracts of *Emblica officinalis* fruit, *Tamarix nilotica* leaf and *Cinnamon* extract on body weight (gm) on diabetic rats from Week 1 to Week 6. Groups: NC-Normal Control, DC-Diabetic Control, MF-Metformin treated, A1-(*Emblica officinalis* fruit extract, 200 mg/kg), A2-(*Emblica officinalis* fruit extract, 400 mg/kg), B1-(*Tamarix nilotica* leaf extract, 200 mg/kg), B2-(*Tamarix nilotica* leaf extract, 400 mg/kg), C1-(*Cinnamon* extract, 200 mg/kg), C2-(*Cinnamon* extract, 400 mg/kg).

3.3. Serum Cholesterol
At the end of the experimental period, the level of serum cholesterol of the diabetic control rats (DC) was significantly (p≤0.05) higher than the normal control (NC). On treatment with the plant extracts a highly significant (p≤0.001) decrease in the serum cholesterol level was observed in a few groups (A1, B2 and C1) in comparison to the diabetic control (DC). There was no significant difference observed among the diabetic control (DC). However, in groups A1, B2 and C1 significantly (p≤0.01) lower cholesterol levels were recorded in comparison to the metformin treated group (MF) (figure 3). There was no significant dose-dependent effect observed between the individual plant extract treatments.
3.4. Serum Triglycerides

At the end of the experimental period, the level of serum triglycerides of the diabetic control rats were significantly (p≤0.001) elevated in comparison to the normal control group (NC). All treated diabetic groups (A1, A2, B1, B2, C1, C2, and MF) showed a highly significant (p≤0.001) decrease in the triglyceride levels when compared to the diabetic group (DC). Further, in groups treated with the plant extracts highly significant (p≤0.001) decrease in the triglyceride levels was observed in comparison to the metformin treated group (MF). Only the groups treated with cinnamon extract (C1,C2) exhibited a dose-dependent effect, the triglyceride level in C2 was highly significantly (p≤0.001) lower than the C1 group. Furthermore, the triglyceride levels in groups A1, A2, B1, B2 were significantly higher than C2 (figure 4).

![Figure 4. Effect of aqueous extracts of Emblica officianalis fruit, Tamarix nilotica leaf and Cinnamon extract on Serum Triglyceride levels diabetic rats from Week 1 to Week 6. Groups: NC-Normal Control, DC- Diabetic Control, MF- Metformin treated, A1-(Emblica officianalis fruit extract, 200 mg/kg), A2-(Emblica officianalis fruit extract, 400 mg/kg), B1-(Tamarix nilotica leaf extract, 200 mg/kg), 2-(Tamarix nilotica leaf extract, 400 mg/kg), C1-(Cinnamon extract, 200 mg/kg), C2-(Cinnamon extract, 400 mg/kg).]
was also significantly lower than groups A1, B1 and B2. There was no significant dose-dependent effect observed between the individual plant extract treatments (figure 5).

Figure 5. Effect of aqueous extracts of *Emblica officinalis* fruit, *Tamarix nilotica* leaf and *Cinnamon* extract on Serum Creatinine level diabetic rats from Week 1 to Week 6. Groups: NC- Normal Control, DC- Diabetic Control, MF- Metformin treated, A1-(*Emblica officinalis* fruit extract, 200 mg/kg), A2-(*Emblica officinalis* fruit extract, 400 mg/kg), B1-(*Tamarix nilotica* leaf extract, 200 mg/kg), 2-(*Tamarix nilotica* leaf extract, 400 mg/kg), C1-(Cinnamon extract, 200 mg/kg), C2-(Cinnamon extract, 400 mg/kg).

4. Discussion

Herbal medicines are the primary form of healthcare known to mankind. Natural products are important sources of antioxidant and anti-cancer lead molecules and this is mainly due to the high degree of diversity and novelty. The increased quest for plant antioxidants and their use in scientific research, as well as industrial purposes, is mainly due to their strong biological activity. Phytocompounds have been proven to be more safe and efficacious with fewer side effects in comparison to the synthetic antioxidants which could possibly be carcinogenic in nature [10, 11]. Obesity and diabetes are chronic metabolic diseases that are associated with risk of hypertension, renal failure, coronary heart diseases, stroke, all being fatal [12, 13]. The present study showed that treatment of STZ rats by various plants extracts improved insulin sensitivity. However, the plant extracts did not induce any anti-obesity effects although another study on other plant based compounds has reported the same [14]. Insulin resistance is strongly associated with metabolic dyslipidemia in diabetes and obesity. The authors’ findings showed that the three plant extracts failed to modulate changes in body weight or gain and lipid profiles as reported previously [14]. The present study showed that treatment of STZ rats by various plants extracts improved insulin sensitivity. *Emblica officinalis*, *Tamarix nilotica* and Cinnamon are few of the most widely acclaimed therapies for diabetes treatment. Their antidiabetic effect is attributed to their insulin secretagogue effect which could be due to the stimulation of the beta cells or regeneration of beta cell functioning by alleviating the oxidative stress. Literature suggests a direct or indirect antioxidant nature of the extracts, which could be due to the free radical scavenging effect of bioactive phenolic components present. Flavonoid and phenolic constituents have been reported from the leaves, roots and flowers of *T. nilotica* (Ehrenb.). Leaves revealed the presence of nilotinins, hirtellins, tamarixinin A, 1,2,6-tri-O-galloyl-D-glucose, methyl ferulate 3-O-sulphate, coniferyl alcohol 4-O-sulphate,kaempferol 4’-methyl ether, tamarixetin and quercetin 3O-beta-D-glucopyranuronide [15, 16, 17]. The amla fruit also contains phenolic compounds, tannins, phyllembelic acid, phyllemblin, rutin, curcuminoids and
embol [18]. It has been suggested that the major active components in cinnamon are water soluble doubly-linked procyanidin type – A polymers which were likely misidentified as methylhydroxychalcone polymer (MHCP) in earlier studies [19]. These polyphenolic compounds act as monomers or oligomers, responsible for in vitro insulin enhancing activity in epididymal fat cells and shown in vitro to have insulin-like activity as well as an antioxidant effect.

The findings of the present study showed that the aqueous extracts of Emblica officinalis, Tamarix nilotica and Cinnamon at both doses used; showed a profound antihyperglycemic effect and ameliorated the associated metabolic disarray, including hyper triacylglycerolaemia and elevated serum creatinine in the diabetic rats which continued with duration of the treatment period. The hypocholesterolaemic effect was however more pronounced in groups treated with lower doses of Emblica fruit extract(A1), Tamarix leaf extract (B1) and a high dose of Cinnamon extract( C2).

In consensus with the findings of the present study, the hypolipidaemic and antidiabetic effect of Emblica officinalis fruit extract has been previously reported [20, 21, 22]. Further, an experimental study on male Wistar rats fed a high-fructose (65 %) diet for 1 week, and treated with an ethyl acetate (EtOAc) extract of amla, a polyphenol-rich fraction, at 10 or 20 mg/kg body weight per day, showed that the extract alleviated the dyslipidaemia caused due to the fructose – induced metabolic syndrome [18]. Similar findings were reported in a study by [23] on diabetic rats treated with a plant extract, powdered rhizome of Curcuma longa (turmeric) and the dried fruits of Emblica officinalis. The treatment achieved significant lowering of plasma glucose and glycated haemoglobin in diabetic rats comparable to that of the glyburide, a sulfonylurea drug, treated group.

The findings in the present study demonstrate an antihyperglycemic and antihyper triacylglycerolaemic effect of the Tamarix leaf extract at both doses used (200 mg/Kg and 400 mg/Kg) while the lower dose was effective in reducing the serum cholesterol level in diabetic rats. These findings explain the antioxidant activity of T. nilotica leaves reported earlier [24, 25]. The in vivo antihyperglycemic and hypoglycemic effect of cinnamon extracts have been widely reported in the past as well in the recent times [26, 27]. The results of the present study show a marked antihyperglycemic effect of the cinnamon extract at both high and low doses used (200 mg/Kg and 400 mg/Kg) in diabetic rats. Further, the cinnamon extracts used also mitigated diabetes associated hypolipidemia and corrected the enhanced serum creatinine levels in diabetic rats. Previous experimental and clinical studies, on the use of cinnamon extract have also postulated that cinnamon does have an antilipidemic effect on diabetic rats [27] and on individuals suffering from type-2 diabetes [26].

Oxidative stress has been widely incriminated in the pathogenesis of diseases such as, diabetes and cardiovascular diseases. Phenolic compounds and flavonoids were described as having antioxidative action in living systems, as they act as scavengers of free radicals [28]. Thus, there is a growing trend of using plant products in therapy as they are rich in these antioxidants. Recent studies suggest that oxidative stress may contribute to the pathogenesis of diabetes counting the β- cell dysfunctioning [29, 30]. Thus, a new strategy for alleviating the oxidative damage in diabetes makes use of natural antioxidants. The non pharmacological management of diabetes includes an appropriate diet management. A myriad of foods like cereals, vegetables and spices have been assessed for their anti-hyperglycemic effect [31, 32, 33, 34, 35] as well as weight gain in experimental as well as clinical studies.

This key findings of the study revealed that the three plant extracts used function as potent antioxidant agents, which could lead to additional health benefits. An overall assessment of the
efficacy of the plant extracts used showed they were comparable to the synthetic drug used. Hypoglycemic herbs are widely used as nonprescription treatment for diabetes. However, few herbal medicines have been well characterized and demonstrated an efficacy in systematic clinical trials as those of synthetic drugs. These herbs may lower blood glucose however; their test results are subject to several factors. Firstly, each herb contains thousands of components, only a few of which may be therapeutically effective. Secondly extraction of active component is not easy (14, 15). Further phytochemical screening and clinical trials using these herbs are imperative.

Conclusion
In conclusion, all the plant extracts used effectively reduced the serum glucose level as the experimental period progressed and demonstrated a marked hypolipidemic effect evidenced by the lower serum triglyceride and total cholesterol levels. The overall effect of the plant extracts was significantly better than the synthetic drug, metformin in terms of antihyperglycemia and anti hyper triacylglycerolaemia. The plant extracts were efficacious at both the doses used except the cinnamon extract which showed a more pronounced effect on all parameters at a higher dose of 400 mg/kg. LDL cholesterol, or total cholesterol levels may benefit from the regular inclusion of cinnamon in their daily diet. Body weight was also restored in diabetic rats on treatment with the plant extracts. The results suggest that the three plant extract studied are potential phytotherapeutic agents which could be used for the management of diabetes type 2 and dyslipidemia associated with it. In addition, Emblica fruit and cinnamon are dietary components and could be beneficial in non-pharmacological management of diabetes mellitus. However, further studies to identify active components in the polyphenol-rich fraction of Emblica and Tamarix should be conducted to elucidate the mechanism of the protective effect against the metabolic syndrome. More experimental studies are however imperative to assess the anti-diabetic effect of Tamarix leaf extract owe to its potent antioxidative potential.

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References:


