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# Searching for anti-hyperglycemic phytomolecules of *Tecoma stans*

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ABSTRACT

*Tecoma stans* plant is well postulated to decrease blood glucose level, but its mode of action and the molecules responsible are still controversial. Thus, the aim of this study was to evaluate the effect of leaves methanol extract of *Tecoma stans* and some of its fractions on starch tolerance in healthy rats, *in vitro* inhibition of α-amylase, and their effects of sub-chronic administration of glucose, lipid pattern, kidney and liver functions and antioxidant status in streptozotocin (STZ) induced diabetic rats. In starch tolerance experiment, both ethyl acetate and crude flavonoids fractions decreased glycemic peak values in healthy rats to extent similar to that of acarbose. In STZ sub-chronic experiment all preparations of *Tecoma stans* significantly decreased fasting glucose with variable degrees. The results indicated that the crude methanol extract had the most antidiabetic potential followed by the methylene chloride rich alkaloid fraction while the crude flavonoids fraction achieved the lowest effect. All *Tecoma stans* different preparations have positive effects on serum lipid pattern, kidney and liver function parameters, in addition to the antioxidant parameters (MDA and GSH) in liver tissues. In conclusion, the present study suggested that the alkaloids synergistically act as antidiabetic agent with other bioactive compounds of *Tecoma stans* especially flavonoids as hypoglycemic agents and the ethyl acetate fraction had the most powerful effects.

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## 1. Introduction

Diabetes mellitus is an endocrine disorder which mainly raises glucose level in the blood stream due to the defect in insulin secretion, insulin action or both [1]. In addition, it is associated with long term damage such as malfunction of eyes, kidneys, nerves, heart and blood vessels. In another word, diabetes is associated with health complications, including renal failure, sexual dysfunction, heart disease, stroke and blindness.

*Tecoma stans* (L.) (Bignoniaceae) is used in traditional medicine for treatment of diabetes and urinary disorder control [2]. Hypoglycemic responses of that plant have been reported by many authors [3-5]. In the past, it had been thought that, tecomine and tecostatine alkaloids were responsible for the antidiabetic potential of the plant [6]. On the contrary, four purified alkaloids from *Tecoma stans* (*T. stans*) including tecomine and tecostanine did not decrease glucose level of diabetic db/db mice [7,8]. In a more recent paper, Tecoma aqueous extract exhibited their antidiabetic action through the inhibition of intestinal α-glucosidase without decreasing fasting glucose in STZ-induced diabetic rats [9]. They showed that phenolic compounds present in the tecoma aqueous extract are responsible for these positive activities.

Now, the antidiabetic activity *of Tecoma stans* is well postulated but its mode of action and the molecules responsible is still controversial. So, searching for the anti-hyperglycemic phytomolecules of *Tecoma stans* is still attractive to attention. As mentioned previously, alkaloids and phenolics are the main compounds thought to be responsible for the hypoglycemic potential of *Tecoma stans*. Therefore, the objective of the current paper was, to separate crude flavonoid and other fractions of successive extraction of methanolic extract and to examine them as follows: the effect on the starch hydrolysis in healthy rats, *in vitro* inhibition of α-amylase, and the effects of sub-chronic doses of methanolic extract and their fractions on glucose, lipid pattern, kidney and liver functions and antioxidant parameters in STZ-induced diabetic rats.

## 2. Experimental

### *2.1. Plant samples*

Fresh leaves of *Tecoma stans* were collected in April 2014 from Agric. Collage Garden of Mansoura University, Mansoura, Egypt. The samples of the plant were shade dried and coarsely powdered.


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### 2.2. Extraction of plant samples

The powdered leaves of *T. stans* were extracted by soaking in methanol overnight [10]. The extraction process was repeated twice, and then the combined methanolic extract was evaporated under vacuum till dryness to obtain a greenish brown extract.

### 2.3. Successive extraction of T. stans leaves extract

Methanolic extract of *T. stans* suspended in water (in a ratio of 1:4) and extracted using four solvents increasing in their polarity i.e. petroleum ether, methylene chloride, ethyl acetate and butanol. Then the solvent of each fraction was removed by evaporation under reduced pressure and stored under cooling till use.

### 2.4. Extraction of crude flavonoids

The separation of the crude flavonoid fraction was carried out according to the method described by Ibrahim [11]. After several steps the crude flavonoids were extracted in $H_2O$ saturated n-butanol. The solvent was evaporated under reduced pressure and stored under cooling till use.

### 2.5. Preliminary phytochemical analysis

Preliminary phytochemical analysis was carried out on crude methanolic extract (as mother liquor) of *T. stans* and its fractions: methylene chloride, ethyl acetate, butanol and flavonoids fractions to detect the presence of terpenes, saponins, alkaloids, flavonoids, glycosides and tannins. All qualitative tests of the aforementioned constituents were carried out according to Harborne [12].

### 2.6. Determination of total flavonoids content

The total flavonoids content of the mother methanolic extract of *T. stans* and their resultant fractions by successive extraction or crude flavonoid extraction were estimated by aluminium chloride colorimetric method as described by Lin and Tang [13]. Methanolic extract of *T. stans* (1 mg) or their resultant fractions were mixed with 0.1 mL of 10% aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After incubation for 40 minutes at room temperature, the absorbance of the developed color was measured at 415 nm. Quercetin (QE) was used as a standard ranged from 0.005 to 0.1 mg/mL and the total flavonoid content was expressed as milligram (QE) per g dry extract.

### 2.7. Determination of total phenolics content

The total phenolics of the previously mentioned fractions were determined according to the Folin-Ciocalteu method as described by Singleton *et al.* [14]. Gallic acid was chosen as a standard ranged from 0.025 to 0.5 mg/mL. The reaction was prepared by mixing 1 mL of methanolic solution (0.3 mg/mL), 9 mL distilled water, 1 mL Folin-Ciocalteu's reagent and 10 mL 7% sodium carbonate. After incubation for 90 minutes at room temperature, the absorbance of reaction mixture was recorded at 765 nm and total phenolics content were expressed as mg gallic per gram dry extract.

### 2.8. HPLC analysis

HPLC chromatography was used for the detection and quantification of different flavonoids and polyphenols in the leaves of *T. stans*. HPLC analysis was conducted in The Laboratories of Food Technology Research Institute, Giza, Egypt.

#### 2.8.1. Identification and quantification of flavonoids

Flavonoids of *T. stans* leaves were identified and quantified by HPLC technique. An Agilent 1100 Series high-performance liquid chromatography equipped with diode array detector was used. Column temperature was set at 35 °C, gradient elution was employed with 50 mM $H_3PO_4$, pH = 2.5 (solution A) and acetonitrile (solution B) as the mobile phase. All flavonoids were quantified using the external standard method and the samples were analyzed in triplicate. The method was described in details by Mattila *et al.* [15].

#### 2.8.2. Identification and quantification of polyphenols

Phenolic compounds were extracted from *T. stans* powder leaves, then identified and quantified according to the method described by Goupy *et al.* [16] using the reversed phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) with a guard column Alltima $C_{18}$, 5 mm (Alltech). A gradient elution was employed using a solvent system of A ($CH_3COOH$ 2.5%), B ($CH_3COOH$, 8%) and C (acetonitrile). The flow rate of the solvent was 1 mL/min and separation was performed at 35 °C. Phenolics were assayed by external standard calibration at 280 nm and expressed in mg/g dry matter.

### 2.9. Thin layer chromatography (TLC)

Crude methanolic extract and its fractions were spotted on TLC plate according to standard method described by Harborne [11]. Rutin was used as a reference flavonoid. Different mobile phases were employed in the screening tests and selected the one in which separation of the bands was clear: acetic acid: formic acid: water (4:1:1, *v*: *v*: *v*). All plates were visualized with vanillin sulfuric acid reagent.

### 2.10. Anti α-amylase activity

The plant methanolic extract (as mother liquor) and its three solvent fractions plus flavonoids fraction were applied to measure the anti α-amylase activity using starch iodine method as described by Hussain *et al.* [17]. Inhibition of α-amylase activity was calculated as follows:

$$\text{Inhibition \%} = (S\text{-}C) \times 100/(B\text{-}C) \quad (1)$$

where, S = absorbance of the sample, B = absorbance of blank (no enzyme) and C = absorbance of control (no starch). $IC_{50}$ % was calculated as the concentration of the sample (µg/mL) which inhibits 50% of α-amylase activity.

### 2.11. Animals

#### 2.11.1. Carbohydrate tolerance curve

Starch (2 g/kg body weight) was administrated orally to healthy adapted rats after giving an oral dose of water, acarbose, methanolic extract, methylene chloride, ethyl acetate, butanol and crude flavonoids fractions as 250 mg/kg body weight. To evaluate in *vivo* inhibitory activity of different fractions of *T. stans* on the hydrolysis of starch, blood was drown for the determination of the levels of blood glucose after 1, 2 and 4 hours.

#### 2.11.2. Sub-chronic biological experiment

A number of 60 albino rats weighing (100-150 g) were kept 7 days for adaptation under laboratory conditions. All rats were fed corn meal diet and allowed free access water. Then, seven rats were housed as a healthy group (Group1).

**Table 1.** Phytochemical screening of *T. stans* methanolic extract and other fractions.

| Compound | Methanol extract | Methylene chloride fraction | Ethyl acetate fraction | Butanol fraction |
|---|---|---|---|---|
| Terpenes | ++ | + | - | - |
| Saponins | + | + | ++ | + |
| Alkaloids | ++ | +++ | - | - |
| Flavonoids | ++ | ++ | +++ | ++ |
| Glycosides | ++ | - | + | +++ |
| Tannins | ++ | - | +++ | - |

* "-": Absent; "+": Low; "++": Moderated and "+++": High.

**Table 2.** Total phenolics and flavonoids contents.

| Compound | Crude methanol | Methylene chloride | Ethyl acetate | Butanol | Crude flavonoids |
|---|---|---|---|---|---|
| Flavonoids mg as quercetin equivalents/g fraction | 51.19 | 39.21 | 59.91 | 45.75 | 55.55 |
| Polyphenols mg as gallic acid equivalents (GAE) /g fraction | 230.3 | 102.49 | 279.41 | 232.0 | 264.7 |

The other fifty three rats were fasted for 24 hours then injected intraperitoneal by freshly prepared streptozotocin in 0.1 M citrate buffer, pH = 4.5 at a dose of 40 mg/kg b.w to induce diabetes mellitus [18]. In order to stave off the hypoglycemic effect during the first day after streptozotocin injection, rats were given 5% glucose solution orally as reported by Orhan *et al*. [19]. After 72 hours of streptozotocin injection, serum glucose levels of all diabetic rats (fasted for 18 hours) were determined. Rats showed blood glucose levels over 250 mg/dL were considered as diabetic rats and were employed in the study. The diabetic rats was then randomly divided into 6 groups (2-7). Each group composed of 7 rats. Group 2 represented control diabetic rats, received normal diet for 28 days without any treatment. Group 3 represented diabetic rats, fed a normal diet for the same period with metformin hydrochloride powder (500 mg/kg b.w.) as a reference drug. Groups 4, 5, 6 and 7 were diabetic rats received normal diet until the end of the experiment with methylene chloride, ethyl acetate, butanol and crude flavonoid fractions, respectively as 250 mg/kg b.w. At the end of the experiment blood samples were taken from eyes orbital plexus and centrifuged without anticoagulant at 4000 rpm for 20 min to separate serum, which kept frozen (-20 °C) till analysis. Then, the rats were fasted overnight, killed by decapitation and livers were removed. Liver samples were then prepared for further determinations.

#### *2.11.3. Biochemical analysis*

Serum total cholesterol (TC) [20], triglycerides (TG) [21], high density lipoprotein cholesterol (HDL-c) [22], glucose [23], creatinine [24], uric acid [25,26] and alanine aminotransferase (ALT) [27] were estimated using enzymatic kits (Spinreact company, Spain). Serum low density lipoprotein cholesterol (LDL-c) was calculated according to the equation of Friedewald *et al*. [22].

LDL-c = Total cholesterol- (Triglycerides/5)-HDL-cholesterol (2)

Serum very low density lipoprotein cholesterol (VLDL-c) was calculated according to Norbert [28] formula: VLDL-c = (Triglycerides/5). Atherogenic index was calculated by using method of Schulpis and Karikas [29] formula: TC-HDL-C/HDL-C

#### *2.11.4. Tissue malondialdehyde (MDA) and glutathione (GSH) assays*

Hepatic lipid peroxidation level was estimated using colorimetric reaction employ thiobarbituric acid-positive reactant substances (TBARS) and was expressed in terms of the malondialdehyde (MDA) concentration using 1,1,3,3-tetraethoxy propane as a standard. Nanomoles of MDA per gram of tissue for the samples under investigation were measured using spectrophotometer at 535 nm [30].

The hepatic reduced glutathione (GSH) level was measured at 412 nm using colorimetric reaction in the presence of 5,5'-dithio-*bis*-2-nitrobenzoic acid (DTNB). Reduced glutathione was expressed as µmole/g tissue using standard curve prepared by known amounts of pure GSH [31].

### *2.12. Statistical analysis*

All values were expressed as the mean± SD of seven animals per group. Data were analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0). Values were considered statistically significant at $p$ <0.05.

## 3. Results and discussion

### *3.1. Phytochemical screening*

The yield for methanol extract was 18.6 g as dark brown dry extract per 100 g dry leaves of *T. stans*. Fractionation of the crude methanolic extract depending on the ability of the compounds to dissolve in such solvent was produced petroleum ether (6.5%), $CH_2Cl_2$ (5.5%), EtOAc (15.5%), *n*-BuOH (23.2%) fractions. Petroleum ether fraction was discarded as it represented hydrocarbons and other fatty materials. On the other hand, the extraction percentage of the separately crude flavonoids extraction from the mother methanolic extract was 11.44 %.

The results of preliminary phytochemical screening of the *T. stans* were recorded in Table 1. The mother methanolic extract revealed the presence of tannins, flavonoids, alkaloids, terpenes, glycosides and traces of saponins. It could be noticed that flavonoids were distributed between methylene chloride, ethyl acetate and butanol fractions. Alkaloids were only detected in methylene chloride fraction. The detected phytochemical components of *T. stans* in the present study agreed with the findings of references [32,33].

### *3.2. Determination of total flavonoids and polyphenols*

Flavonoids are a large group of the phenolic compounds consisting mainly of flavonols and anthocyanins. Phenolic compounds can prevent body cells and organs from injuries by hydrogen peroxide, damage by lipid peroxides and scavenging free radicals [34]. The total flavonoids were determined in different solvent extracts as mg of quercetin per gram of dry extract. Total flavonoids content in Table 2 ranged from 39.21 to 59.91 mg as quercetin/g in methylene chloride and ethyl acetate solvents fractions, respectively.

Polyphenols secondary plant metabolites are integral part of human and animal diets [35]. The results showed that ethyl acetate solvent fraction contained the highest amount of polyphenols as 279.41 mg gallic acid equivalents (GAE)/g dry fraction while methylene chloride had the lowest one 102.4941 mg gallic acid equivalents/g dry fraction.

**Table 3.** HPLC analysis for *T. stans* flavonoids and polyphenols (mg/100 g dry leaves).

| Compound | Concentration (mg/100 g dry leaves) | Compound | Concentration (mg/100 g dry leaves) |
|---|---|---|---|
| Naringin | 21.4 | Chlorogenic acid | 17.04 |
| Rutin | 112.7 | *p*-Hydroxybenzoic acid | 6.28 |
| Rosmarinic acid | 27.1 | Caffeic acid | 1.40 |
| Quercitrin | 16.6 | Vanillic acid | 5.87 |
| Quercetin | 14.0 | Ferulic acid | 31.38 |
| Kaempferol | 2.8 | Isoferulic acid | 5.02 |
| Apigenin | 3.9 | Ellagic *acid* | 8.91 |
| Hesperetin | 7.97 | 3,4,5-Trimethoxy-cinnamic acid | 21.56 |
| 7-OH-flavone | 0.9 | *p*-Coumaric acid | 1.49 |
| Catechin | 10.74 | Cinnamic acid | 0.31 |
| Gallic acid | 0.9 | Protocatechuic *acid* | 11.01 |
| Pyrogallol | 29.0 | | |

**Table 4.** In vivo starch tolerance in orally administrated rats.

| Time | 0 min | 60 min | | 120 min | | 240 min | |
|---|---|---|---|---|---|---|---|
| Groups | Glucose (mg/dL) | Glucose (mg/dL) | % Reduction | Glucose (mg/dL) | % Reduction | Glucose (mg/dL) | % Reduction |
| Normal | 105.22 | 117.76 | -11.91 | 127.8 | -21.45 | 115.21 | -9.49 |
| Acarbose | 102.11 | 124.47 | -21.89 | 94.41 | 7.54 | 75.88 | 25.68 |
| Crude methanol extract | 93.86 | 109.12 | 7.33 | 88.64 | 5.56 | 82.80 | 11.78 |
| Methylene chloride | 106.13 | 117.90 | -11.09 | 90.17 | 15.03 | 93.30 | 12.08 |
| Ethyl acetate | 92.88 | 100.50 | -8.20 | 72.70 | 21.72 | 77.30 | 16.77 |
| Butanol | 121.15 | 129.11 | -6.75 | 111.27 | 8.15 | 98.29 | 18.86 |
| Crude flavonoids | 106.91 | 113.0 0 | -5.69 | 82.81 | 22.54 | 73.90 | 30.87 |

It could be notice from the previous results that extraction solvent mainly affect on the total content of extracted phenolics. However, the separation of crude flavonoid fraction by the method described by Reference [11] did not increase extracted total flavonoid content in crude flavonoid fraction which may due to polarity varying of flavonoids content, existence of other extracted molecules and method qualification.

### *3.3. HPLC fractionation of flavonoids and polyphenols contents*

Table 3 showed identification and determination of flavonoids and other phenolics components of *T. stans* leaves by HPLC. Twenty three compounds could be identified, the phenolic profile showed the presence of phenolic acid derivatives (gallic acid, pyrogallol, protocatchuic, chlorogenic, *p*-OH benzoic, vanillic, ferulic, isoferulic, caffeic acid, ellagic acid, *p*-coumaric, 3,4,5-trimethoxy cinnamic acid and cinnamic acid, three flavonoid glycosides (rutin, naringin and quercitrin) and six free flavonoids (quercetin, kampferol, apigenin, hesperitin, 7-OH flavones and catechin) in different concentrations. Rutin was the predominant phenolic (112.7 mg/100 g dry leaves) followed by ferulic, pyrogallol, rosemarinic, 3,4,5-methoxy cinnamic, naringin, chlorogenic, quercitrin and quercetin (31.38, 29.00, 27.1, 21.56, 21.4, 17.04, 16.6 and 14.0 mg/100 g dry leaves), respectively. The previously mentioned phenolics of *T. stans* leaves with high or moderated concentrations were reported to have different biological activities such as anti-hyperglycemic, antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, anti-hyperlipidemic, antiproliferative, and anti-cancer activities [36-40].

### *3.4. TLC technique for T. stans flavonoids*

Thin layer chromatography (TLC) was performed to separate different spots of *T. stans* methanolic extract as well as all other fractions using the running system (ethyl acetate: formic acid: water, 4:1:1, *v*:*v*:*v*) as the most appropriate solvent system. Rutin; the predominant detected flavonoid in *T. stans*; was used as reference flavonoid. TLC chromatogram revealed that flavonoid fraction has clear rutin band, while a weak band of rutin appeared in crude methanol, ethyl acetate and butanol fractions. On the opposite rutin was absent in methylene chloride fraction. From the above mentioned results, it could be easily concluded that rutin was well extracted in crude flavonoid fraction.

### *3.5. Anti α-amylase activity*

#### *3.5.1. In vitro experiment*

Glycosidases i.e. α-amylase are a group of enzymes which responsible for the digestive hydrolysis of food starch and so they cause an increase in blood glucose level after a personal meal. So in vitro experiment for antiα-amylase activity was done to evaluate the effect of *T. stans* methanolic extract as well as all other fractions as natural inhibitors for α-amylase activity as alternative strategy in the control of blood glucose level. The results indicated that flavonoids rich fractions i.e. crude flavonoids and ethyl acetate fractions had strong in vitro anti α amylase activity. $IC_{50}$ of α-amylase for crude flavonoids, ethyl acetate, crude methanol, butanol and methylene chloride fractions were 801.28, 803.85 and 853.24, 1026.69 and 1207.72 µg/mL, respectively.

#### *3.5.2. In vivo experiment*

Starch tolerance experiment was conducted to assure in vivo antiα-amylase activity of different preparations of *T. stans* after administration of an oral dose of starch solution (2 g/kg b.w) to rats groups. It could be seen from Table 4 and Figure 1 that crude flavonoid rich fraction had the strongest postprandial anti-hyperglycemic effect in starch tolerance test. It decreases the glycemic peak by 22.54 and 30.87% after 2 and 4 hours of orally administration of starch. In other word, our findings demonstrated that rutin rich crude flavonoid fraction was more effective to decrease starch tolerance curve. In this respect [41] stated that rutin metabolites are capable of inhibiting *α*-glucosidase activity both in vivo and in vitro experiments. Moreover, it inhibits the formation of protein glycation end products which correlates with complications of diabetes [42].

### *3.6. Sub-chronic experiment*

It could be noticed from Table 5 that blood glucose level of all diabetic groups significantly decreased with different extends of oral injection of different preparations of *T. stans* comparing to diabetic untreated rats (Group 2).

**Table 5.** Glucose levels of different STZ treated rat groups.

| Groups * | Glucose level | | |
|---|---|---|---|
| | 0 day (mg/dL) | 28 day (mg/dL) | % Reduction |
| Normal | 93.55 | 107.08$^{f}$ | - |
| Diabetic rats | 299.67 | 287.33$^{a}$ | - |
| Diabetic rats + Metformin hydrochloride (as a reference drug) | 311.11 | 198.27$^{b}$ | 36.27 |
| Diabetic rats + Crude methanol extract | 324.17 | 121.17$^{e}$ | 62.60 |
| Diabetic rats + Methylene chloride fraction | 322.86 | 127.81$^{d,e}$ | 60.40 |
| Diabetic rats + Ethyl acetate fraction | 288.54 | 135.87$^{d}$ | 52.91 |
| Diabetic rats + Butanol fraction | 311.76 | 134.37$^{d}$ | 56.89 |
| Diabetic rats + Crude flavonoids fraction | 281.90 | 149.68$^{c}$ | 46.90 |
| Least Significant Difference (0.01) | - | 12.2138 | - |
| Least Significant Difference (0.05) | - | 9.1548 | - |

* Means±SE with different letters superscripts (a, b, c, d, e, f) in the same column are significant at $p < 0.05$ using one way ANOVA test.

Crude methanol extract showed strongest antidiabetic activity with 62.6% reduction in blood glucose level followed by rich alkaloids-methylene chloride fraction 60.41%. Blood glucose values were 324.17 and 322.86 mg/dL at the beginning of the experiment and significantly decreased to 121.17 and 127.81 mg/dL for the same groups at the end of the experiment, respectively. Administration of crude flavonoid fraction of *T. stans* to diabetic rats caused the lowest decrease in blood glucose level (46.90%).

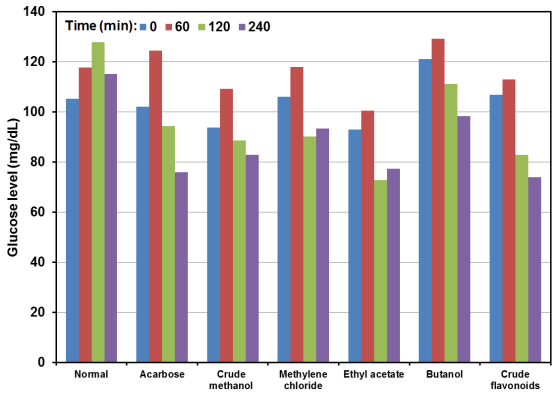


**Figure 1.** Starch tolerance curve of different preparations of *T. stans*.

Our findings for the antidiabetic potency of *T. stans* preparations agreed to a large extent with those obtained by Reference [5,6]. The significant anti diabetic potential of ethyl acetate, butanol and methylene chloride fractions of *T. stans* as shown in Table 5 may be due to the presence of hypoglycemic flavonoids, alkaloids, saponins and terpenes. It could be suggested that these biomolecules synergistically improve the sensitivity of insulin receptor to insulin or stimulate pancreatic β-cells to release insulin which may finally lead to improvement of carbohydrate hydrolyzing enzymes towards the re-establishment of normal blood glucose level.

On the other hand, high antidiabetic potency achieved by alkaloid rich preparations (crude methanol extract and methylene chloride fraction) of *T. stans* in the present study were different from those of other researchers , who showed that four purified alkaloids from *Tecoma stans* including tecomine and tecostanine did not decrease glucose level of diabetic db/db mice [7,8]. Here, again we suggest that tecomine and tecostanine alkaloids synergistically act with other bioactive molecules such as flavonoids in *T. stans* as hypoglycemic agents.

In the present investigation it was mentioned that flavonoid-rich fraction decreased fasting blood glucose with a percent of 46.90 in STZ diabetic rats experiment. This finding disagreed with reference [9] that showed that rich phenolics aqueous extract of *Tecoma stans* reduces triglycerides and cholesterol, without modifying fasting glucose in STZ-induced diabetic rats. This may due to the differences in phenolics content between the examined fractions. Generally, flavonoids exert their hypoglycemic effects either through their capacity to inhibit glucose absorption or to increase glucose tolerance [43]. In this respect, rutin; the predominant detected flavonoid in the present study; was recently reported as a strong hypoglycemic agent since it decreased blood glucose level of diabetic patients [44]. The mode of action of rutin was shown by several researchers. They stated that, it is a polyphenolic flavonoid, which could induce pancreatic β cells to produce insulin and or protect them from further deterioration [36,45,46]. Although, flavonoids fraction positively affect on glucose level in STZ treated rats in the present study. The results showed that mother methanolic extract and their resultant fractions exert more hypoglycemic effect than that of the previously mentioned fraction. In other words, the previously results which were indicated that crude flavonoids fraction were the most effective fraction as α-amylase inhibitors in both in vivo and in vitro experiments were not confirmed by their pattern in STZ-treated rats. The present data prove that flavonoids of *T. stans* act synergistically with other natural metabolites especially alkaloids as anti-hyperglycemic agents in the treatment of diabetes.

Tables 6 and 7 showed blood lipid profile of experimental animals. It could be noticed that diabetic rats showed a significant elevation of total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol. Their values were 103.37, 162.14, 32.42 and 40.4 mg/dL, respectively, at the end of feeding period. Also, diabetic control rats showed a significant decrease in HDL-cholesterol where their value was 30.55 mg/dL. High Atherogenic Index (AI) 2.38 was recorded for the diabetic rats at the end of the experiment.

These findings agreed with those of reference [47], who mentioned that diabetes mellitus (especially Type 1) is accompanied by hypercholesterolemia, hyperlipidemia and hepatic steatosis. Insulin deficiency in diabetes patients leads to abnormal metabolic processes; this in turn leads to accumulation of lipids such as TG and TC [48].

Also, Suckling and Jackson [49] reported that insulin deficiency will lead to decrease activity of lipoprotein lipase and increase free fatty acids mobilisation from peripheral fat depots. So, the STZ-induced diabetic rat is thus considered as an animal model of Type 1 diabetes mellitus and hyperlipidemia.

Total cholesterol content of diabetic rats treated by all plant extracts decreased significantly; comparing to diabetic untreated rats (Group 2) at the end of feeding period. Moreover, administration of oral doses of methylene chloride, crude methanol and ethyl acetate fractions to experimental animals for 28 days decreased total cholesterol to a non-significant value with normal rats, where their values were 70.68, 71.44 and 72.72 mg/dL, respectively.

Concerning serum triglycerides, the lowest value was obtained when ethyl acetate fraction of *T. stans* daily administrated to diabetic rats for 28 day, it decreased to be 60.91 mg/dL. Treatment with other fractions of *T. stans* significantly decreased serum triglycerides with different extents.

**Table 6.** Serum total cholesterol, triglycerides and HDL-cholesterol of different STZ treated rat groups (n=7).

| Groups * | Total cholesterol (mg/dL) | Triglycerides (mg/dL) | HDL-c (mg/dL) |
|---|---|---|---|
| Normal | 73.58 $^{d}$ | 50.83 $^{g}$ | 45.98 $^{a}$ |
| Diabetic rats | 103.37 $^{a}$ | 162.14 $^{a}$ | 30.55 $^{g}$ |
| Diabetic rats + Metformin hydrochloride (as a reference drug) | 91.79 $^{b}$ | 144.85 $^{b}$ | 34.15 $^{e,f}$ |
| Diabetic rats + Crude methanol extract | 71.44 $^{d}$ | 84.97 $^{e}$ | 35.02 $^{d,e}$ |
| Diabetic rats + Methylene chloride fraction | 70.68 $^{d}$ | 110.19 $^{c}$ | 32.77 $^{f,g}$ |
| Diabetic rats + Ethyl acetate fraction | 72.72 $^{d}$ | 60.91 $^{f}$ | 39.56 $^{c}$ |
| Diabetic rats + Butanol fraction | 82.96 $^{c}$ | 96.70$^{d}$ | 37.90$^{d}$ |
| Diabetic rats + Crude flavonoids fraction | 86.78 $^{c}$ | 84.27 $^{e}$ | 42.13 $^{b}$ |
| Least Significant Difference (0.01) | 6.2665 | 10.4704 | 3.3379 |
| Least Significant Difference (0.05) | 4.6970 | 7.8480 | 2.5019 |

* Means±SE with different letters superscripts (a, b, c, d, e, f) in the same column are significant at $p < 0.05$ using one way ANOVA test.

**Table 7.** Serum VLDL- cholesterol, LDL- cholesterol and atherogenic index of different STZ treated rat groups *.

| Groups | VLDL-c (mg/dL) | LDL-c (mg/dL) | AI (Atherogenic index) |
|---|---|---|---|
| Normal | 10.16 $^{g}$ | 17.44 $^{c,d}$ | 0.60 $^{f}$ |
| Diabetic rats | 32.42 $^{a}$ | 40.40 $^{a}$ | 2.38 $^{a}$ |
| Diabetic rats + Metformin hydrochloride (as a reference drug) | 28.97 $^{b}$ | 38.67 $^{b}$ | 1.68 $^{b}$ |
| Diabetic rats + Crude methanol extract | 16.99 $^{e}$ | 19.43 $^{c}$ | 1.03 $^{d}$ |
| Diabetic rats + Methylene chloride fraction | 22.03 $^{c}$ | 15.88 $^{d}$ | 1.15 $^{c}$ |
| Diabetic rats + Ethyl acetate fraction | 12.18 $^{f}$ | 20.98 $^{c}$ | 0.83 $^{e}$ |
| Diabetic rats + Butanol fraction | 19.34 $^{d}$ | 25.72 $^{b}$ | 1.18 $^{c}$ |
| Diabetic rats + Crude flavonoids fraction | 16.85 $^{e}$ | 27.80 $^{b}$ | 1.05 $^{d}$ |
| Least Significant Difference (0.01) | 2.0941 | 4.4498 | 0.1337 |
| Least Significant Difference (0.05) | 1.5696 | 3.3353 | 0.1002 |

* Means ± SE with different letters superscripts (a, b, c, d, e, f, g) in the same column are significant at $p < 0.05$ using one way ANOVA test.

**Table 8.** The concentration of serum creatinine, uric acid and ALT *.

| Groups | Creatinine (mg/dL) | Uric acid (mg/dL) | ALT (U/L) |
|---|---|---|---|
| Normal | 1.20 $^{e}$ | 0.95 $^{g}$ | 36.62 $^{f}$ |
| Diabetic rats | 3.14 $^{a}$ | 4.65 $^{a}$ | 66.50 $^{a}$ |
| Diabetic rats + Metformin hydrochloride (as a reference drug) | 2.55 $^{b}$ | 3.24 $^{b}$ | 49.80 $^{b}$ |
| Diabetic rats + Crude methanol extract | 1.70 $^{c,d}$ | 1.89 $^{c}$ | 41.85 $^{d,e}$ |
| Diabetic rats + Methylene chloride fraction | 1.80 $^{c,d}$ | 1.71 $^{d}$ | 43.65 $^{c,d}$ |
| Diabetic rats + Ethyl acetate fraction | 1.54 $^{d}$ | 1.25 $^{e,f}$ | 39.37 $^{e,f}$ |
| Diabetic rats + Butanol fraction | 1.84 $^{c}$ | 1.47 $^{e}$ | 46.20 $^{b,c}$ |
| Diabetic rats + Crude flavonoids fraction | 1.87 $^{c}$ | 1.24 $^{f,g}$ | 43.66 $^{d,e}$ |
| Least Significant Difference (0.01) | 0.2711 | 0.3134 | 5.4390 |
| Least Significant Difference (0.05) | 0.2032 | 0.2349 | 4.0768 |

* Means± SE with different letters superscripts (a, b, c, d, e, f, g) in the same column are significant at $p< 0.05$ using one way ANOVA test.

Similarly the lowest values for VLDL-c and atherogenic index were obtained when ethyl acetate fraction of *T. stans* daily administrated to diabetic rats for 28 day, it decreased to be 12.18 and 0.83 mg/dL, respectively. Also, other fractions of *T. stans* gave satisfied results for the same mentioned parameters.

Lipid profile of animal groups treated with different *T. stans* preparations; especially ethyl acetate fraction; was relative to that of healthy non diabetic rats. It was clear that ethyl acetate fraction; the most successful fraction in controlling lipid profile of diabetic rats; had the highest amount of flavonoids as (59.91 mg as quercetin equivalents/g). Therefore, it could be suggested that the positive effects of different preparations of *T. stans* in controlling lipid profile might due to the presence of considerable amount of flavonoids (39.21 to 59.91 mg as quercetin equivalents/g fraction). In this respect, Glässer *et al.* [50] and Lee *et al.* [51] reported that some flavonoid components had inhibitory effect on hepatic cholesterol biosynthesis.

It could be noticed from Table 3 that, ferulic acid was the most abundant polyphenols (31.38 mg/100 g dry leaves) in *T. stans* leaves. Moreover, different preparations of *T. stans* had high polyphenols content which ranged from 102.49 to 279.41 as gallic acid/g dry extract. Therefore, it was suggested that, total polyphenols content of *T. stans* especially, ferulic acid may contribute the anti-hyperlipidemic action in diabetic rats. These findings agreed with those of reference [52] who showed that ferulic acid was able to decreased abnormal lipid levels (i.e. free fatty acids, cholesterol, triglycerides and phospholipids) of nicotine-induced tissue damage rats.

Table 8 summarizes the concentration of serum creatinine, uric acid and an alanine aminotransferase (ALT) which were found to be significantly increased in diabetic non treated rats when compared to normal control rats. Their values were 3.14 mg/dL, 4.65 mg/dL and 66.50 U/L, respectively, at the end of feeding period. When different extracts of *T. stans* were administered to rats, the above parameters were significantly decreased with the superior of ethyl acetate fraction which their values were 1.54, 1.25 mg/dL and 39.37 U/L, respectively.

Table 9 showed the effect of *T. stans* leaves extracts on liver malondialdehyde (MDA) and reduced glutathione (GSH) levels. It could be observed that, MDA level as an index of lipid peroxidation, significantly increased in livers of streptozotocin diabetic animals (Group2; 42.14nmole of MDA /g tissue) as compared to healthy rats (Group1; 18.77 nmole of MDA /g tissue). Oxygen free radicals intensively increased in diabetic animals due to impaired glucose metabolism, which leads to oxidative stress as mentioned by Ceriello *et al.* [53]. Treatment with different preparations of *T. stans* resulted in significant decrease in liver tissue MDA. The lowest value of MDA was achieved when rats treated orally with the mother methanol extract (25.11 nmole/g tissue).

It was observed that GSH level; which protect the body cells against toxic effects of lipid peroxidation; was significantly depleted in liver tissue of diabetic control animals (Group 2; 22.42 µmole/g tissue) as compared to healthy rats (Group 1; 50.5 µmole/g tissue). Treatments with *T. stans* different preparations for 28 days resulted in significant increase in liver tissue GSH. Its values were 46.22, 38.22, 45.54, 40.13and 41.11 µmole/g tissue, for groups of rats treated with methanol, methylene chloride, ethyl acetate, butanol and flavonoids fractions, respectively.

In the current study, high polyphenolic contents (Table 2) of *T. stans* preparations which ranged from 102.49 to 279.41 gallic acid/g dry extract may be responsible for the high antioxidant activity.

**Table 9.** Liver malondialdehyde (MDA) and reduced glutathione (GSH) levels.

| Groups | MDA (nmole/g tissue) | GSH (µmol/g) |
|---|---|---|
| Normal | 18.77 [f] | 50.50 [a] |
| Diabetic rats | 42.14 [a] | 22.42 [e] |
| Diabetic rats + Metformin hydrochloride(as a reference drug) | 37.52 [b] | 28.65 [d] |
| Diabetic rats + Crude methanol extract | 25.11 [e] | 46.22 [b] |
| Diabetic rats + Methylene chloride | 32.84 [c] | 38.22 [c] |
| Diabetic rats + Ethyl acetate | 27.88 [e] | 45.54 [b] |
| Diabetic rats + Butanol | 30.37 [c] | 40.13 [c] |
| Diabetic rats + Crude flavonoids | 29.12 [c,d] | 41.11 [c] |
| Least Significant Difference (0.01) | 4.9444 | 5.5802 |
| Least Significant Difference (0.05) | 3.7061 | 4.1826 |

* Means± SE with different letters superscripts (a, b, c, d, e, f, g) in the same column are significant at $p < 0.05$ using one way ANOVA test.

In this respect, Pietta [54] showed that the antioxidant activity of the polyphenolic flavonoids may due to their ability to neutralize the free radicals and reduce their formation. The same author added that most digested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability. Both the absorbed flavonoids and their metabolites may display an *in vivo* antioxidant activity.

## 4. Conclusions

In conclusion, all preparations of *T. stans* are able to exhibit positive effects as antihyperglycemic, anti-hyperlipidemic, hepatorenal protective and antioxidant roles with different extents. Crude flavonoids had the strongest post-prandial antihyperglycemic effect in starch tolerance curve in normal rats. The concentration of crude flavonoids which give 50% inhibition of α-amylase was the lowest between all the preparations of *T. stans.* All examined fractions of *T. stans* in the present study were able to decrease fasting blood glucose significantly with different extends in STZ sub-chronic experiment. The maximum effect was detected when rats were treated with rich alkaloids fractions (methanolic and methylene chloride fraction). The present study suggests that alkaloids synergistically act with other phytomolecules especially flavonoids as anti-hyperglycemic agents. Generally, the anti-diabetic effect of *T. stans* is due to intestinal α-glucosidase inhibition by decreasing the postprandial hyperglycaemia peak, in addition to modifying fasting glucose. Finally ethyl acetate fraction exhibits the most desirable effects on lipid pattern, antioxidant and hepato-renal blood parameters.

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## References

[1]. Veeramani, C.; Pushpavalli, G.; Pugalendi, K. V. *J. Appl. Biomed.* **2008**, *6*, 19-26.
[2]. Hernandez-Galicia, E.; Aguilar-Contreras, A.; Aguilar-Santamaria, L.; Roman-Ramos, R.; Chavez-Miranda, A. A.; Garcia-Vega, L. M.; Flores-Saenz, J. L.; Alarcon-Aguilar, F. J. *Proc. West Pharmacol. Soc*. **2002**, *45*, 118-124.
[3]. Perez, G. R. M.; Ocegueda, A. Z.; Munoz, J. L. L.; Avila, J. G. A.; Morrow, W. W. *J. Ethnopharmacol.* **1984**, *12,* 253-262.
[4]. Meckes-Lozoya, M.; Mellado-Campos, V. *J. Ethnopharmacol.* **1985**, *14*, 1-9.
[5]. Naranjo, D. L. P.; Salvado, M. C. J.; Jimenez, G. R.; Menendez, T. M. F.; Perez-Santoya, P. E. *Rev. Cubana Med. Milit.* **2003**, *32(1)*, 13-17.
[6]. Aguilar, L. C.; Macias, S.; Chagoya, A.; Cardenas, A.; Diaz, P.; Cantu, J. M. *Fitoterapia* **1993**, *64*, 304-305.
[7]. Costantino, L.; Lins, A. P.; Barlocco, D.; Celotti, F.; ElAbady, S. A.; Brunetti, T.; Maggi, R.; Antolini, L. *Pharmazie* **2003**, *58*, 140-142.
[8]. Costantino, L.; Raimondi, L.; Pirisino, R.; Brunetti, T.; Pessotto, P.; Giannessi, F.; Lins, A. P.; Barlocco, D.; Antolini, L.; El-Abady, S. A. *Il Farmaco* **2003**, *58*, 781-785.
[9]. Aguilar-Santamaria, L.; Ramirez, G.; Nicasio, P.; Alegria-Reyes, C.; Herrera-Arellano, A. *J. Ethnopharmacol.* **2009**, *124*, 284–288.
[10]. Adaramoye, O.; Amanlou, M.; Habibi-Rezaei, M.; Pasalar, P.; Ali, M. M. *Asian Pac. Trop. Med.* **2012**, *5*, 427-433.
[11]. Ibrahim, G. S.; Abdulmumin, K. Y.; Musa Yaro, A. H. *J. Pharmacol. Toxicol.* **2008**, *3,* 351-356.
[12]. Harborne, J. B. Phytochemical Methods, 2nd ed., Chapman and Hall publications, London, New York, 1988.
[13]. Lin, J. Y.; Tang, C. Y. *Food Chem.* **2007**, *101*, 140-147.
[14]. Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. *Method. Enzymol.* **1999**, *299,* 152-178.
[15]. Mattila, P.; Astola, J.; Kumpulainen, J. *J. Sci. Food. Agric.* **2000**, *48*, 5834-5841.
[16]. Goupy, P.; Hugues, M.; Boivin, P.; Amiot, M. J. *J. Sci. Food Agric.* **1999**, *79,* 1625-1634.
[17]. Hussain, S. J.; Tsujiyama, I.; Takasugi, M.; Islam, M. A.; Biswas, R. S.; Aoshima, H. *Food Sci. Technol. Res.* **2008**, *14,* 261-268.
[18]. Ghasemi, M.; Sadeghipour, H.; Asadi, S.; Dehpour, D. *Life Sci.* **2007**, *81,* 960-969.
[19]. Orhan, D.; Aslan, M.; Orhan, D. D.; Ergun, F.; Yesilada, E. *J. Ethnopharmacol.* **2006**, *108,* 280-286.
[20]. Rosclau, P.; Bernt, E.; Gruber, W. *Zklin. Chem. Klin. Biochem.* **1974**, *12,* 403-407.
[21]. Schettler, G.; Nussel, E. *Sozialmed Praeventivmed* **1975**, *10,* 25-29.
[22]. Friedewald, W. T.; Robert, I. L.; Donald, S. F. *Clin. Chem.* **1972**, *18,* 499-502.
[23]. Trinder, P. *Ann. Clinc. Biochem.* **1969**, *6,* 24-27.
[24]. Murray, R. L. Creatinine In: Clinical Chemistry; Theory, Analysis and Correlation, Kaplan, L. A. and Pesce, A.J. (Eds.), CV Mosby Company, St. Louis, 1984, pp. 1247-1253.
[25]. Fossati, P.; Prencipe, L.; Berti, G. *Clin Chem.* **1980**, *26,* 227-231.
[26]. Schultz, A. *Clin Chem.* **1984**, *418,* 1261-1266.
[27]. Henry, R. J.; Chiamori, N.; Golub, O. J.; Berkman, S. *Am. J. Clin. Pathol.* **1960**, *34,* 381-398.
[28]. Norbert, W. T. Clinical Guide to Laboratory Tests. Third ed. Saunders W. B. Company; Philadelphia, 1995.
[29]. Schulpis, K.; Karikas, G. A. *Paediatrics* **1998**, *101,* 861-864.
[30]. Draper, H. H.; Hadley, M. In: Parcker, L. & Glazer, A. ed. Method. Enzymol. New York, Academic Press, 1991, v. 186 (B), pp. 421-443.
[31]. Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82,* 70-77.
[32]. Mohamed, Z. M. S.; Yousry, M. G.; Camacho, L. M.; El-Shanhorey, N. A.; Salem, A. Z. M. *Afr. J. Microbio Res.* **2013**, *7,* 418-426.
[33]. Dash, S.; Das, C.; Sahoo, D. C.; Sahoo, A. C. *Nat. Pharm. Technol.* **2011**, *1,* 5-8.
[34]. Ahmad, N.; Mukhtar, H. *Nutr. Rev.* **1999**, *57*, 78-83.
[35]. Velioglu, Y. S.; Mazza, G.; Gao, L. *J. Agric. Food Chem.* **1998**, *46(10)*, 4113-4117.
[36]. Kamalakkannan, N.; Prince, P. S. *Basic. Clin. Pharmacol. Toxicol.* **2006**, *98,* 97-103.
[37]. Oke-Altuntas, F.; Aslim, B.; Duman, H.; Gulpinar, A. R.; Kartal, M. *J. Food Biochem.* **2015**, *39,* 409-416.
[38]. Ou, S.; Kwok. *J. Sci. Food Agric.* **2004**, *84,* 1261-1269.
[39]. Bumrungpert, A.; Pingeesakikul, T.; Tirawanchai, N.; Tuntipopipat, S.; Lilitchan, S.; Komindr, S. *Fed. Am. Soc. Exp. Bio. J.* **2012**, *26*, 263-267.
[40]. Khan, M. T.; Lampronti, I.; Martello, D.; Bianchi, N.; Jabbar, S.; Choudhuri, M. S.; Datta, B. K.; Gambari, R. *Int. J. Oncol.* **2002**, *21,* 187-192.
[41]. Adisakwattana, S.; Yibchok-Anun, S.; Charoenlertkul, P.; Wongsasiripat, N. *J. Clin. Biochem. Nutr.* **2011**, *49*, 36-41.
[42]. Pashikanti, S.; de Alba, D. R.; Boissonneault, G. A.; Cervantes-Laurean, D. *Free Radical Bio. Med.* **2010**, *48,* 656-663.
[43]. Brahmachari, G. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, 1st edition, Chapter 6, Research Signpost, 2011, pp. 187-212.
[44]. Sattanathan, K.; Dhanapal, C. K.; Umarani, R.; Manavalan, R. *J. Appl. Pharma. Sci.* **2011**, *1,* 227-231.

[45]. Vessal, M.; Hemmati, M.; Vasie, M. *Comp. Biochem. Physiol. Toxicol. Pharmacol.* **2003**, 135-357.
[46]. Coskun, O.; Kanter, M.; Korkmaz, A. and Oter, S. *Pharmacol Res.* **2005**, *1*, 117-123.
[47]. Brixova, E. *Folia, Fac. Med. Univ. Comenian. Bratis.* **1981**, *19*, 9-90.
[48]. Goldberg, R. B. *Diabetes Care.* **1981**, *4*, 561-572.
[49]. Suckling, K. E.; Jackson, B. *Prog. Lipid. Res.* **1993**, 32, 1-24.
[50]. Glasser, G.; Graefe, E. U.; Struck, F.; Veit, M.; Gebhardt, R. *Phytomedicine* **2002**, *9,* 33-40.
[51]. Lee, J. Y.; Park, H. R.; Moon, S. O.; Kwon, Y. J.; Rhee, S. *J. Food Sci. Biotechnol*. **2004**, *2,* 176-184.
[52]. Adluri, R. S.; Nagarajan, D.; Periyaswamy, V.; Venugopal, P. M. *Fund. Clin. Pharmacol*. **2008**, *22*, 557-567.
[53]. Ceriello, A.; Quatraro, A.; Giugliano, D. *Diabet. Med.* **1992**, *9,* 297-299.
[54]. Pietta, P. G. *J. Nat. Prod.* **2000**, *63,* 1035-1042.