

## Herbal Treatment for Type 2 Diabetes Compared to Current Medicines

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### Abstract

**Objective:** To study the effect of herbal treatment for type 2 diabetes compared to current medicines.

**Method:** Total of 24 Locally bred male Albino Wistar rats weighing 180 to 200gm, purchased from research institute of Agha Khan University Karachi Pakistan were used throughout the experiments. These rats were divided into 4 groups containing 6 rats in each group: Control (C), Model (M), Test 1 (T1) and Test 2 (T2). C was given as 0.9% saline solution. M and both test groups were treated with streptozotocin (STZ) as 30mg/kg body weight intraperitoneally for 4 weeks (twice a day) to produce the hyperglycemic effects. After 4 weeks, rats were fasted for 16 hours and with one drop of blood from tail plasma glucose was checked by using glucometer. After STZ treatment T1 group treated with metformin 100mg/kg body weight given orally and T2 with a self-prepared 100mg tablets of black pepper powdered in extraction of small new sprouting leaves of *Azadirachta indica* (Neem tree) twice a day for three weeks. Behavioral activity was noted. After 3 weeks of treatment rats were decapitated, and the blood collected for biochemical activity. Statistical Package for Social Sciences (SPSS) v.20 was used for statistical analysis.

**Results:** Results show that locomotor activity of test 2 group of rats increases as compare to test 1 and model group. Similarly, stimulatory activity of rats also increases. Results also indicates that anxiety of test 2 group of rats decreases and recognition memory non-significantly increases in test 2 group of rats as compare to test 1 and model groups. Biochemical parameters including random plasma glucose and serum cholesterol were significantly ( $P<0.05$ ) decreased in test 2 group of rats as compare to test 1 and model group of rats.

**Conclusion:** Herbal treatment with metformin and modification of lifestyle were highly effective means of delaying or preventing type 2 diabetes. It was also concluded that herbal treatment for type 2 diabetes is one of the treatment option which not only prevent the development of complications but substantially reducing the individual and public health burden of diabetes.

**Keywords:** *Azadirachta indica*; Black Pepper; Type 2 Diabetes

### Introduction

With the changes of lifestyle and living environment, diabetes mellitus is spreading around the world at an alarming rate. The prevalence of diabetes is rising through worldwide, but the situation is particularly disquieting in Asia. It reports for 90% to 95% of all diabetic subjects globally with the preponderance of them being 45-64 years old [1,2]. As reported by International Diabetes Federation (IDF), 425 million adults are surviving with diabetes [3]. This number is expected to raise to around 642

million or one in ten adults by 2040. Diabetes and its related micro and macro vascular complications not only decrease the quality of people's lives but also bring about enormous economic and social burdens [4]. Previous study also indicates that the incidence of type 2 diabetes was diminished by 58 percent with the lifestyle intervention and by 31 percent with metformin. The comprehensive lifestyle intervention was as impressive in older participants as it was in younger participants [5]. Type 2 Diabetes subjects are generally autonomous of exogenous insulin, but when their blood glucose levels are not well controlled with diet alone or with oral hypoglycemic drugs, they may need it.

Control of modifiable risk factors, including blood pressure, lipid levels and levels of glycaemia can ameliorate some, but not all, of the excess disease burden. Randomized controlled trials of glucose lowering in Type 2 diabetes have demonstrated benefits on reducing microvascular and macro-vascular complication rates [6]. Subjects with type 2 diabetes are at elevated risk for a number of serious health problems, including cardiovascular disease, premature death, blindness, kidney failure, amputations, fractures, frailty, depression, and cognitive decline. In prospective epidemiologic studies, the incidence of many of these outcomes is directly associated with the degree of hyperglycemia, as measured by the plasma glucose or the glycated hemoglobin level, a measure of the mean blood glucose level during the previous 2 to 3 months.

Thus, after adjustment for other risk factors, an increase of 1% in the glycated hemoglobin level is associated with an increase of 18% in the risk of cardiovascular events, an increase of 12 to 14% in the risk of death, and an increase of 37% in the risk of retinopathy or renal failure [7]. The diseases seriously threatened the public health [8]. The present study, therefore, aims to introduce the one of the herbal treatment option for type 2 diabetes compared to current medicines. Currently, clinical treatment mainly focuses on symptom intervention via pharmacological agents, the side effects of which, especially for long-term usage, however, have resulted in major concerns to the public [9]. Alternative therapy with less side effects is urgently needed.

## Method

Locally bred male Albino Wistar rats weighing about 180 to 200gm on arrival purchased from animal house, research institute of Agha Khan University Karachi Pakistan were used throughout the experiments. The rats were housed individually in specially designed cages with saw dust cover floor in a quiet room, with free access to cubes of standard rats' food and water for at least 3 to 4 days before starting the experiment so that rats could adapted them to the new environment. In this study 24 rats were used, divided into 4 groups containing 6 rats in each group: control, model, test 1 and test 2. Control was given as 0.9% saline solution.

### Model and Test Group Treatment by Streptozotocin (Stz)

Model and both test groups of rats were treated with streptozotocin (STZ) as 30mg/kg body weight given intraperitoneally for 4 weeks (twice a day) to produce the hyperglycemic effects. After 4 weeks of treatment. Rats were fasted for 16 hours. One drop of blood was collected from tail vein by venipuncture and plasma glucose was checked by using glucometer. These readings were noted.

**Test 1 Group Treatment:** After STZ treatment this group was treated with metformin 100mg/kg body weight given orally for three weeks twice a day.

**Test 2 Group Treatment:** After STZ treatment this group was treated orally with a self-prepared 100mg tablets of mixture of equal amount of black pepper powdered in extraction of small new sprouting leaves of *Azadirachta indica* (Neem tree). Tablet was given 100mg/kg body weight twice a day for three weeks.

**Preparation of Saline:** For the preparation of saline 0.9gm of NaCl was taken and make up the volume up to 100ml of water.

**Preparation of Metformin:** 100 mg tablet of metformin was taken. At the time of use each tablet was crushed and dissolved in 0.9% saline solution and given orally to test 1 group of rats.

**Preparation of Plant Drug:** A self-prepared 100mg tablets of mixture of equal amount of black pepper powdered in extraction of small new sprouting leaves of *Azadirachta indica* (Neem tree) were prepared. Each tablet at the time of use was dissolved in 0.9% saline solution and given orally to test 2 rats.

**Preparation of Extracts:** Total of 200 new sprouting leaves of *Azadirachta indica* (Neem tree) washed, dried and crushed. These leaves were grinded in a local grinder machine used in home with 0.9% saline solution. Volume of saline water was used to obtain slightly thin paste.

## Handling

Experimental rats require extensive handling for oral administration of drugs and such animals should be handled on a regular basis in non-life threatening situations like weighing, petting, giving food treats. This makes the animals respond positively to handling and learn to recognize individuals. To remove the rat from cage, it is picked up by the tail close to the base and placed on the flat surface of a laboratory bench. While still holding to the tail with the right thumb and forefinger, the scruff of the animal is reached for with your thumb and forefinger, positioning them firmly on either side of the animal's head at the level of the mandible. Simultaneously, the rest finger and palm of the left hand are used to firmly press the thorax or trunk down against the flat surface of the bench. There after the scruff (the loose skin over the neck) is gathered between the thumb and the forefinger and used to lift up the animal by the scruff. The tail may be held either firmly against the trunk with the fifth finger of the left hand or left hanging free. When held firmly this way, the rat is restrained and the esophagus is as straight as possible.

## Behavioral Techniques

After one week of plant extracted compounds administration behavioral activities were monitored. The open field apparatus for locomotor activity was used in this experiment consist of a square area (76\*76cm) with walls 42cm high and floor divided into 25 equal squares. The experiment was performed under day light, in a very quiet room to avoid any noise effect. An animal taken out from the cage and placed in the center square of the apparatus, rats move from the center square, crossing with all four paws, corner sitting,

grooming, gnawing, these all activities scored for 5 minutes. Home cage (26×26×26) with saw dust cover floor was used as the no of cage crossing and increasing intensities of grooming and gnawing were monitored. Anxiety of rats was monitored with the help of Light and Dark box which consisted of two compartments. Both the compartments were of same size (26×26×26 cm), with a door (12 cm×12 cm) between the compartments. One compartment was made up of black plastic walls while other with transparent plastic. Time spent in the light box was monitored for 5 minutes. The activity in this box was determined in a lighted place(360lux) using a 60W white light bulb. Noval object recognition test was used to assess recognition ability of rats. The apparatus used was an open field box (40×40×40 cm) made of gray painted wood. The floor was covered with saw dust. The objects to be discriminated were two similar transparent glasses filled with white cement (A1 and A2) and a metallic container of same size filled with white cement (new object, B). The test comprised of three phases; 1) Habituation phase, 2) Training phase and 3) Test phase. On the 1st day each rat was habituated to the open field box without any object for 15 min. On the 2nd day each rat was placed in the open field for 15 min and allowed to freely explore two identical objects (two glasses filled with cement). On the 3rd day, during the test phase, one old object used during the training session was replaced by novel object (B) and rat was left to explore the objects for 5 min. Both objects presented during the test session were different in texture, color and size. Recognition index was calculated by the ratio  $TB/(TA+TB)$  [TA=Time spent exploring the familiar object A; TB=Time spent exploring the novel object B].

### Decapitation of Rats

After 3 weeks of treatment rats were decapitated, by a specialized kit for dissection careful removal of fur under the neck, the jugular veins were exposed. The veins were then punctured with a clean and sharp syringe and the blood collected in the gel containing tube and then tube placed in an incubator for 30 minutes and the serum collected after centrifugation on a bench centrifuge at 300g for 15 min. Blood also collected in an anticoagulant containing tube to get plasma.

### Diagnostic Analyzer

Plasma was separated by centrifuge at  $3500 \times g$  for 10 minutes. Roche Hitachi 902 analyzer was used for biochemical tests.

### Statistical Analysis

Statistical Package for Social Sciences (SPSS) v.20 was

used for statistical analysis. Results were represented as mean,  $\pm$  SD (n=6) significant difference by Tukey HSD and Duncan test \* $P < 0.05$ , considered statistically significant level from C, M, T1 and T2 following one-way ANOVA.

## Results

Figure 1(a) Effects of self-prepared tablet treatment on open field behavior shows effects on control, model and test groups of rats. Statically analyzed by one-way ANOVA (df4, 23) ( $F=5.2345$ ) (\* $p < 0.002$ ) shows that after 3 weeks of treatment motor activity was markedly increased in both test 1 and 2 groups of rats as compare to control and model group of rats. (b) Home Cage activity statically analyzed by one-way ANOVA (df4,23) ( $F=11.2371$ ) (\* $p < 0.0003$ ) shows that after treatment stimulator activity (walks in seconds) was markedly increased in both the tests groups of rats as compare to model group but decreased as compare to control group.

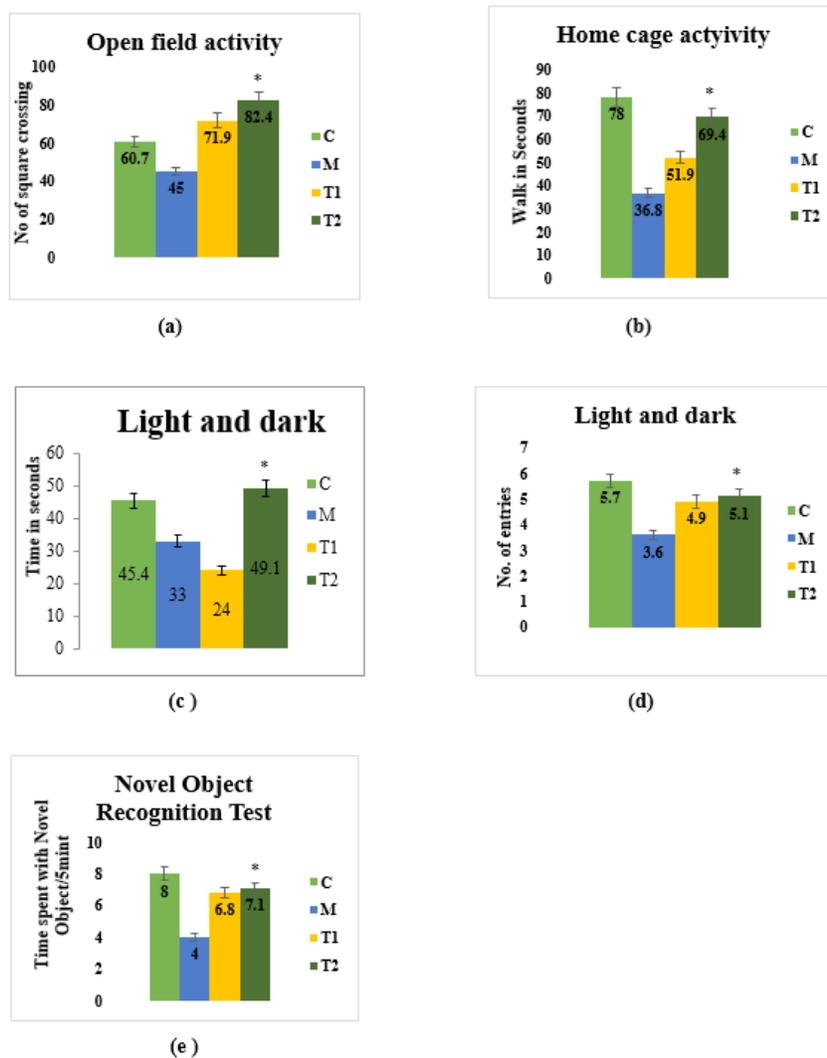
Figure 1(c) Light and Dark activity (time in seconds) statically analyzed by one-way ANOVA (df4,23) ( $F=5.6758$ ) (\* $p < 0.002$ ) shows that anxiety significantly decrease as compare to model, but slightly decreases to control and in test 2 groups but in test 1 group it was increased as compare to model and control group of rats. (d) Entries in light portion statically analyzed by one-way ANOVA (df4,11) ( $F=6.543$ ) (\* $P < 0.003$ ) shows that anxiety markedly decreases in control and both test groups as compare model group of rats.

Figure 1(e) Novel Object Activity statically analyzed by one-way ANOVA (df4,23) ( $F=4.3334$ ) (\* $p < 0.56$ ) shows that recognition memory of test 2 groups was decreased as compare to control but increases as compare to model and test 1 group of rats.

Figure 2 shows the level of fasting plasma glucose in C, M, T1 and T2 after streptozotocin treatment checked by glucometer. Statically analyzed by one-way ANOVA (df4,23) ( $F=3.6218$ ) ( $p < 0.06$ ) shows that fasting plasma glucose level of control group was in normal range but in model, test 1 and tests 2 group increased value of fasting plasma glucose was observed, shows the hyperglycemic effects.

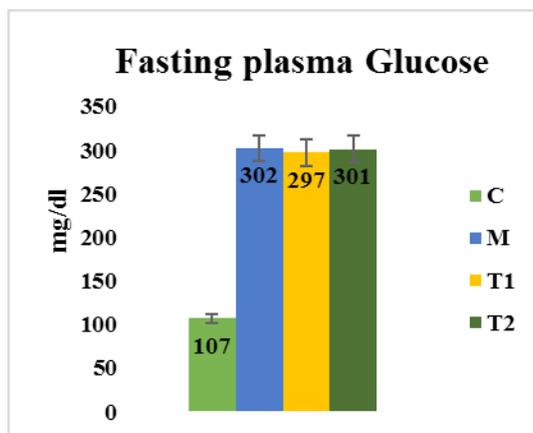
Figure 3(a) Statically analyzed by one-way ANOVA (df4,23) ( $F=9.4555$ ) ( $p < 0.01$ ) shows that random plasma glucose level of tests 2 group decreases significantly as compare to test 1 and model group, and slightly remained equal to control group. (b) Statically analyzed by one-way ANOVA (df4,23) ( $F=5.781$ ) (\* $P < 0.002$ ) shows that total cholesterol level of test 2 group was significantly decreased as compare to model group while, in test 1 group slightly decreases as compare to model group.

## Graphs

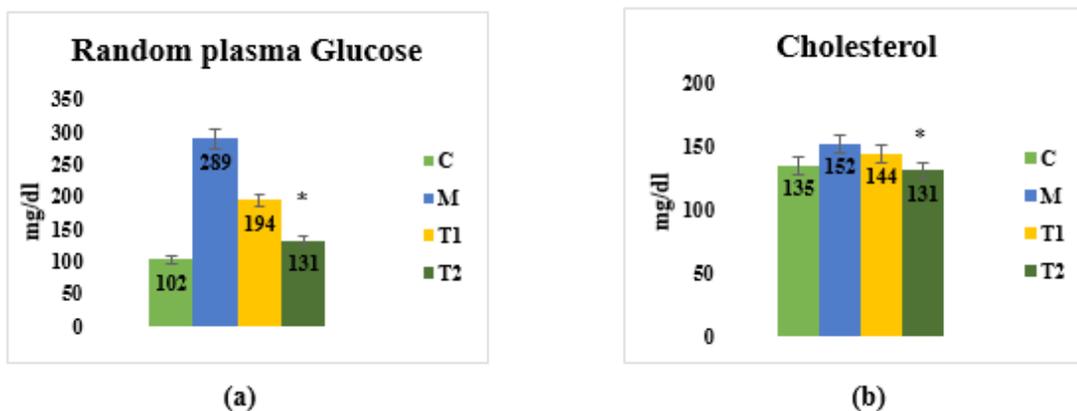


**Figure 1:** Comparison of Behavioral Studies in Control, Model, Test 1 and Test 2 treated rats. Behavioral studies. Results were represented as mean,  $\pm$  SD (n=6) significant difference by student t-test  $p < 0.05$  level from C, M, T1 and T2 following one-way ANOVA. (a) open field activity (no. of square crossing) (b) Home cage activity (walks in seconds) (c) light and dark activity (time in seconds) (d) light and dark activity (entries in light portion) (e) Novel object recognition (time spent with novel object/5 mint).

## Comparison of Biochemical Studies



**Figure 2:** Fasting plasma glucose level in Control, Model, Test 1 and Test 2 rats before decapitation. Biochemical Studies. Effect of fasting plasma glucose levels. Results were represented as mean  $\pm$  SD (n=6) significant difference by student t test  $p < 0.05$  level from C, M, T1 and T2 following one-way ANOVA.



**Figure 3:** Comparison of random plasma glucose level and total cholesterol in Control, Model, Test 1 and Test 2 treated rats. Biochemical Studies. Results were represented as mean  $\pm$  SD (n=6) significant difference by student t test  $p < 0.05$  level from C, M, T1 and T2 following one-way ANOVA. (a) Effect of random plasma glucose level (b) Effect of total cholesterol.

## Discussion

In this study, herbal treatment of black pepper powdered in extraction of small new sprouting leaves of *Azadirachta indica* (Neem tree) show that overall random plasma glucose level and cholesterol level were significantly decreased in test 2 group of rats. The aim was to bring forward the new therapy strategies and cost-effective intervention trials of type 2 diabetes. This treatment can be considered as one of the treatment option because of its ability to control plasma sugar level. As it was previously reported that herbal treatment has no side effects or having minute side effects compared to the allopathic medicines [10]. By comparing with the previous X-ray crystallographic studies of N-terminal catalytic domain of maltase-glucoamylase (ntMGAM) in complex with a new class of  $\alpha$ -glucosidase inhibitors derived from natural extracts of *Salacia reticulata*, a plant used traditionally in Ayurveda medicine for the treatment of type 2 diabetes. Included in these extracts are the active compounds salacinol, kotalanol, and de-O-sulfonated kotalanol [11]. It results that the most potent ntMGAM inhibitor reported to date is de-O-sulfonated kotalanol. It was also reported that it is 2000-fold better than the compounds currently used in the clinic, from which the potential of the salacinol class of inhibitors were highlighted as future drug candidates [11].

Lifestyle changes and treatment with metformin were also studied previously that both reduced the incidence of diabetes in persons at high risk [12]. The lifestyle intervention was more effective than metformin [13]. Current results support the hypothesis that type 2 diabetes can be prevented or delayed at high risk for the disease. Another study shows that the incidence of diabetes was reduced by 58 percent with the lifestyle intervention and by 31 percent with metformin, as compared with placebo [14]. Other study shows that the dried powder of the rhizome of *Curcuma longa* L. (Zingiberaceae) has been used for centuries as a medicinal agent and specifically as an antidiabetic drug. It also indicates that both the rhizome extracts with its principal component curcumin extensively studied [15]. However, studies over the past decade have indicated that curcumin-free turmeric components also possess antidiabetic properties [16]. Similar to our study, which shows that black pepper in extract of *Azadirachta indica* also have antidiabetic properties.

## Conclusion

This study concludes that herbal treatment with metformin and modification of lifestyle were highly effective means of delaying or preventing type 2 diabetes. It was also concluded that herbal treatment for type 2 diabetes is one of the treatment option which not only prevent the development of complications but substantially reducing the individual and public health burden of diabetes.

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