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# Evaluation Of Hypoglycemic And Antihyperglycemic Effects Of Acacia tortilis Seed Extract In Normal And Diabetic Rats

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**Abstract :** Hypoglycemic and antihyperglycemic effect of seed extract of *Acacia tortilis* was evaluated in normoglycaemic and alloxan-induced (135 mg/Kg body weight, i.p) diabetic rats. The extract (200 mg/kg body weight) significantly lowered the blood glucose levels to an extent comparable to that produced by standard Oral hypoglycaemic drug (Gliclazide 22 mg/Kg body weight) in both normal and diabetic rats. The results suggest that seed extract of *Acacia tortilis* possess significant antidiabetic activity.
**Keywords**: Hypoglycemic, Antihyperglycemic effect, *Acacia tortilis*, Alloxan monohydrate, Antidiabetic activity, Wistar rats.

## INTRODUCTION

In India, Ayurvedic medicine is reported to have been successfully used in the treatment of diabetes mellitus. As an alternative mode of treatment, Ayurvedic medicine has been claimed to be less toxic and more efficacious. In accordance to the recommendations of WHO Expert Commitee on diabetes mellitus, an investigation of antihyperglycemic agents of plant origin used in traditional medicine seems important[2]. Many herbs and plant products have shown to have antihyperglycemic effect[3-7].

Now in recent years there has been a growing trend to evaluate the plant constituents on pharmacological basis and screening of more effective and safe hypoglycemic and antihyperglycemic agents. Therefore the present work have been planned to study indigenous plants (*Acacia tortilis*) using new and sensitive technologies and statistical analysis. Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, first described from Africa by Linnaeus in 1773. Acacia is one of the plants that have been frequently used as medicine like Acacia Bark has been used in treatment of haemoptysis, gonorrhea[8], leprosy and headaches[9]. Acacia Catechu has been used for treating fever, diarrhoea, leucorrhoea, piles, erysipelas[10] and throat infection[11]. It is reported that Acacia catechu has hypoglycemic activity[12] hepatoprotective, antipyretic and digestive properties[13,14]. Considering the above facts, the present study was undertaken to evaluate the hypoglycemic and antihyperglycemic activities of *Acacia tortillis* in experimental animal models.

## MATERIALS AND METHODS

### ANIMALS

The study was conducted on healthy albino rats of either sex weighing 150-250 gm. The animals were made available in the Central animal house, GSVM Medical College,Kanpur. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light /dark cycle, at room temperature 25± 3º C and 35-60% humidity), fed in standard pellet diet and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee, Kanpur. The animal care and handling was done as per the guidelines set by the Indian National Science Academy New Delhi, India.

### INDUCTION OF EXPERIMENTAL DIABETES

Diabetes was induced in albino rats of either sex by a single intraperitoneal injection of aqueous alloxan monohydrate (135 mg/kg body weight)[15]. Blood samples were collected before and after the administration of alloxan to know the status of diabetes. After two days, diabetes was confirmed by testing blood glucose level using glucometer and they were further maintained for four days for well establishment of diabetes. The animals with blood glucose level more than 200 mg/dl (moderate diabetes) were selected for the experiment.

### PREPARATION OF PLANT EXTRACT

Seeds of *Acacia tortilis* were taken for the study. Dried seeds of the plant were properly grinded and sieved with mesh size 40-60. Extraction of powdered seed was done with distilled water to separate to volatile and non-volatile fraction with the help of klevenger apparatus and heating mental at 100° C. Non-volatile fraction was cooled and further precipitated with the help of ethyl alcohol for isolation of gum and other solutes. Isolated gum was purified with the help of filtration technique followed by ion exchange and freeze-drying process. Finally, the *Acacia tortilis* seed extract (ATE) was stored in refrigerator until use.

### ACUTE ORAL TOXICITY TEST IN RATS

After an overnight fast of 18 h, ATE was administered orally in doses of 50,100,200,500 and maximum dose of 1000 mg/Kg body weight to groups of rats (n = 6) and closely observed for the first 2-3 hr for signs of toxicity. The behavioral changes and percentage mortality were noted beginning with 24 h up to a period of 14 days[16].

### EXPERIMENTAL DESIGN

The rats were classified into five groups (n = 6).The Group I and II were normal and Group III -V were diabetic. The Animals in Group I (NC) were administered with 1 ml sterile water and served as normal control. Rats of Group II (NATE) were treated with ATE at a doses of 200 mg/Kg, rats of diabetic Group III (DC) treated with 1 ml sterile water served as diabetic control, rats of diabetic Group IV (DATE) were treated with ATE at a doses of 200 mg/kg and rats of diabetic Group V (DG) were treated with 22 mg/kg dose of Gliclazide and served as positive control. The animals of all groups were received the doses orally for 13 consecutive days and at the end of the experimentation an Oral Glucose Tolerance Test (OGTT) [17] and blood glucose estimation was done in all groups.

### ORAL GLUCOSE TOLERANCE TEST

After overnight fasting, 0 min blood samples (0.2 ml) were taken from the all groups by orbital sinus puncture[18]. Glucose solution (2 g/kg of 25% w/v) was administered orally in OGTT. Three more samples were taken at 30 min, 60 min and 120 min after glucose administration.

### BLOOD GLUCOSE ESTIMATION

Blood samples (0.2 ml) were collected in fluoride vials, from orbital sinus puncture with capillary tube under aseptic conditions. Blood samples were then centrifuged at 3000 rpm for 10 min. The clear supernatant serum was taken for estimation of blood glucose level. The plasma blood glucose levels were determined by using GOD–POD method[19]. Span diagnostic reagent kit (code no. B 0112) was used for estimation of blood glucose level.

### STATISTICAL ANALYSIS

All the values of blood glucose and metabolic changes parameters were expressed as mean ± SEM. Data were analyzed statistically using the student's t-test. In all cases, $P<0.05$ was used as the criterion of statistical significance.

**RESULTS**

**ACUTE TOXICITY STUDY**

There were no signs of toxicity, behavioral changes and mortality recorded up to a dose of 1000 mg/kg body weight of the extract.

**EFFECT ON NORMAL RATS**

The effect of *Acacia tortilis* on blood glucose levels in fasting (0 min) and after OGTT was assessed at 30, 60 and 120 min in normal rats. The percentage of blood glucose was observed to be decreased with extract at all the studied time points were 10.34%, 8.67%, 12.11% and 11.51% respectively as compared to normal control. However these values of blood glucose level at 0,30 and 120 min were observed to significantly decreased ($P < 0.05$) whereas at 60 min this effect was highly significant ($P > 0.01$**) [Table 1].**

**EFFECT ON ALLOXAN INDUCED DIABETIC RATS**

The effect of 13-day administration of ATE on blood glucose levels of alloxan induced diabetic rats is shown in Table 2. It is noteworthy to mention that the percentage of blood glucose reduction was much more at 120 min i.e. 30.21% as compare to rest of the studied hrs which were found to be 8.46% at 0 min, 7.24% at 30 min and 9.30% at 60 min. In statistical point of view, it was significantly decreased ($P < 0.05$) at 0 and 60 min .The higher level of significance was found at 30 min ($P < 0.01$) with more pronounced effect at 120 min ($P > 0.001$).

The Gliclazide treated diabetic rats also showed a significant reduction from 254.16 to 134.66 mg/dl (47.02%) at 0 min, from 286.16 to 183.66 mg/dl (35.82%) at 30 min, from 345.83 to 227.66 mg/dl (34.17%) at 60 min and from 350.83 to 183.33 mg/dl (47.74%) at 120 min .The observation at all time points were highly significant ($P > 0.001$). Though the antihyperglycemic activity of the ATE treated rats were significant when compared to control but it was not as effective as gliclazide**. [Table 2]**

**METABOLIC CHANGES IN DIABETIC RATS**

The effect of extract on the body weight changes for two weeks of diabetic rats are shown in **Table 3**. It was observed that in diabetic rats, there were weight loss, relative to day 0, i.e. before the start of the treatment. The diabetic control group rats lost 18.16% of their body weight. The loss was 10.63% ($P <0.05$) for ATE, significantly lower as compared to control. The diabetic rats treated with Gliclazide also showed a body weight reduction of 6.52%, which is significantly much lesser than control diabetic rats. The diabetic control group rats had moderate polyphagia and polydipsia by the end of week 2 of experiment with respective increase in food and fluid intakes of 59.09 and 22.16%. However ,in the presence of ATE the food intake was reduced significantly to 33.43% by day 7 and 53.68% by day 14($P <0.05$) as compared with diabetic control rats. A similar reduction of 35.26% in week 1 and 57.42% in week 2 was observed for gliclazide, the standard drug **[Table 4]**.The Fluid intake decreased by 34.49% ($P <0.05$) in rats treated with ATE. Diabetic rats treated with gliclazide also showed a significant lower water intake of 46.20% ($P <0.05$) as compared to control **[Table 5]**.

**Table 1- Effect of *Acacia tortilis* seed extract in normoglycemic rats**

| S.N. | Groups | Serum glucose level ( mg/dl) | | | |
|---|---|---|---|---|---|
| | | **0 min(Fasting)** | **30 min** | **60 min** | **120 min** |
| **1** | **NC** | 83.83±2.60 | 103.50±2.59 | 96.33±2.92 | 84.00±3.10 |
| **2** | **NATE** | 75.16±2.60[a] (10.34%) | 94.50±1.76[a] (8.69%) | 84.66±1.82[b] (12.11%) | 74.33±2.70[a] (11.51%) |

**Statistical comparison**
Normal control (NC) V/S *Acacia tortilis* seed extract (NATE)
**Values are expressed as Mean ± S.E (% reduction); (n=6)**
**Significance levels**
[a] $P \leq 0.05$ [b] $P \leq 0.01$ [c] $P \leq 0.001$

**Table 2- Effect of *Acacia tortilis* seed extract in alloxan induced diabetic rats**

| S.N. | Groups | Serum glucose level ( mg/dl) | | | |
|---|---|---|---|---|---|
| | | **0 min(Fasting)** | **30 min** | **60 min** | **120 min** |
| **I** | **DC** | 254.16±6.12 | 286.16±5.33 | 345.83±8.10 | 350.83±6.71 |
| **2** | **DATE** | 232.66±4.31 [a] (8.46%) | 265.43±5.94 [b] (7.24%) | 313.66±4.37 [a] (9.30%) | 244.83±3.13 [c] (30.21%) |
| **3** | **DG** | 134.66±3.35 [c] (47.02%) | 183.66±4.72 [c] (35.82%) | 227.66±3.81 [c] (34.17%) | 183.33±3.50 [c] (47.74%) |

**Statistical comparison**
Diabetic control (DC) V/S *Acacia tortilis* seed extract (DATE)
Diabetic control (DC) V/S Gliclazide (DG)

**Values are expressed as Mean ±S.E (% reduction); (n=6)**
**Significance levels**
[a] P ≤0.05 [b] P≤0.01 [c] P≤0.001

**Table 3 -Body weight change in alloxan induced diabetic rats**

| S.N. | Groups | Body weight changes in gm | | |
|---|---|---|---|---|
| | | **Day 0** | **Day 7** | **Day 14** |
| **1** | **DC** | 220.85±2.84 | 200.80±2.34 (9.07%) | 180.74±2.26 (18.16%) |
| **2** | **DATE** | 216.32±3.70 | 204.03±3.35 (5.68%) | 193.32±2.23 [a] (10.63%) |
| **3** | **DG** | 208.62±3.73 | 201.82±3.90 (3.25%) | 195.25±1.85 [a] (6.52%) |

**Statistical comparison**
Diabetic control (DC) V/S *Acacia tortilis* seed extract (DATE)
Diabetic control (DC) V/S Gliclazide (DG)

**Values are expressed as Mean ±S.E (% reduction); (n=6)**
**Significance levels**
[a] P ≤0.05 [b] P≤0.01 [c] P≤0.001

**Table 4 - Food intake changes in alloxan induced diabetic rats**

| S.N. | Groups | Food intake (gm/rat/week) | | |
|---|---|---|---|---|
| | | **Day 0** | **Week 1** | **Week 2** |
| **1** | **DC** | 15.50±1.15 | 21.65±0.55 (-39.67%) | 24.66±0.41 (-59.09%) |
| **2** | **DATE** | 16.30±1.05 | 10.85±0.45 (33.43%) | 7.55±0.45 [a] (53.68%) |
| **3** | **DG** | 16.42±0.92 | 10.63±0.25 (35.26%) | 6.99±0.35 [a] (57.42%) |

**Statistical comparison**
Diabetic control (DC) V/S *Acacia tortilis* seed extract (DATE)
Diabetic control (DC) V/S Gliclazide (DG)

**Values are expressed as Mean ±S.E (% reduction); (n=6)**
**Significance levels**
[a] P ≤0.05 [b] P≤0.01 [c] P≤0.001

**Table 5 - Water intake changes in alloxan induced diabetic rats**

| S.N. | Groups | Water intake (ml/rat/week) | | |
|---|---|---|---|---|
| | | Day 0 | Week 1 | Week 2 |
| 1 | DC | 52.51±4.25 | 58.82±2.80 (-12.01%) | 64.15±2.82 (-22.16%) |
| 2 | DATE | 62.01±1.92 | 50.05±1.75 (19.28%) | 40.62±1.95 [a] (34.49%) |
| 3 | DG | 65.62±2.65 | 48.32±2.52 (26.36%) | 35.30±1.62 [a] (46.20%) |

**Statistical comparison**
Diabetic control(DC) V/S *Acacia tortilis* seed extract(DATE)
Diabetic control(DC) V/S Gliclazide (DG)

**Values are expressed as Mean ±S.E (% reduction); (n=6)**
**Significance levels**
[a] P ≤0.05 [b] P≤0.01 [c] P≤0.001

**DISCUSSION**

Our study has detected the antidiabetic activity of ATE in alloxan induced diabetic rats. Alloxan is a well-known diabetogenic agent widely used to chemically induce Type II diabetes in experimental animals[20].Alloxan and its reduction product dialuric acid establish a redox cycle with formation of superoxide radicals, hydrogen peroxide and lastly highly reactive hydroxyl radicals. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells[21].The Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content[22]. The Moderate diabetic animals are recommended for use in testing drugs for use in Non insulin dependent diabetes mellitus[23]. Hence in this research, moderate diabetes was produced by alloxan monohydrate in a dose of 135 mg/kg body weight.

In light of results, the study indicates that extract of acacia at dose of 200 mg/kg exhibited significant and consistent reduction in blood glucose levels in normoglycemic and diabetic rats. The anti-hyperglycemic effect of aqueous extract might be due to stimulatory effect on the remnant β-cells to secrete more insulin or from regenerated β-cells or increase the glucose uptake. This effect is comparable to that produced by standard drug, Gliclazide (22 mg/kg). However, it was less efficacious than gliclazide. It is well established that sulfonylureas cause hypoglycemia by stimulating insulin release from pancreatic β cells[24]. The comparable effect of the extract with gliclazide suggests the possibility of a similar mode of action.

Another observation arising from this study is the effect of the ATE on the body weight in the treated rats. Insulin deficiency causes drastic elevation in levels of blood glucose as a result of excessive production of endogenous glucose and also causes a drastic change (reduction) in body weight[25], which may be due to excessive breakdown of tissue proteins and lipids caused by insulin insufficiency. The improvement in body weight in diabetic rats treated may be due to improvement in metabolic activity of the system to maintain glucose homeostasis. The other probable mechanism of this benefit might be due to its effect in controlling muscle wasting by controlling the disintegration process. These results suggested that ATE might contains some biological principle(s) that posses insulin protective or insulin-like activity but this hypothesis would require experimental validation and further experiments are required to elucidate the exact mechanism of action as well as on the isolation of bioactive principles.

The metabolic disturbances were corrected after the plant extract was administered for two weeks as shown by a reduction in polyphagia and polydipsia in diabetic rats treated with plant extract.

Although the present findings confirm the hypoglycemic and antihyperglycemic potential in the seeds of *Acacia tortilis*, the precise mechanism of its action requires further studies for appropriate elucidation.

## CONCLUSION

The ATE has antidiabetic activity as it lowers serum glucose levels in diabetic rats and significantly increases glucose tolerance. It also increases body weight of diabetic rats. Further studies are necessary to substantiate the above observation and to work out the exact mechanism of action involved in the antidiabetic activity of this plant.

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