**IJPSR (2011), Vol. 2, Issue 4** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 January, 2011; received in revised form 18 February, 2011; accepted 25 March, 2011

# COMPARATIVE STUDY OF *AZADIRACHTA INDICA* LEAF POWDER AND ITS DIFFERENT EXTRACTS ON GLYCEMIC, INSULINEMIC AND LIPIDEMIC STATUS OF TYPE 2 DIABETIC MODEL RATS

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**Keywords:**

Hypoglycemic, Antilipidemic, Streptozotocin, *Azadirachta indica*, type 2 diabetic rat

**Abbreviations:**

STZ = Streptozotocin
OGTT = Oral glucose tolerance test
TG = Triglyceride
ELISA = Enzyme linked immunosorbent assay
SPSS = Statistical Package for Social Science
ANOVA = Analysis of variance
FSG = Fasting serum glucose
WC = Water control

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**ABSTRACT**

The present study was carried out to investigate the effect of oral feeding of *A. indica* leaf (powder, aqueous and 80% ethanol extract) in STZ induced type 2 diabetic rats. Diabetes was induced by single intraperitoneal injection of STZ to 48hrs old pups and experiments were carried out three months later. For chronic experiment type 2 rats were divided into 5 groups: 1) Water control, 2) Glibenclamide (5gms/kg b.w.) treated, 3) *A. indica* powder [mixed with rat feed (1.25 g/kg b.w.)], 4) Aqueous extract and 5) 80% ethanol extract treated. The test drugs were used at a dose of 1.25 g/kg b.w. for 28 consecutive days. Body weight was checked every week. Blood was collected by cutting the tail tip at the beginning and on the days 7, 14, 21 and by decapitation on the 28$^{th}$ day. Results of the experiment showed that *A. indica* leaves exerted a gradual reduction in serum glucose level of type 2 diabetic rats. A significant reduction in serum glucose level was noticed on the 21 day ($p = 0.001$ for powder and $p = 0.001$ for ethanol extract) as well as on 28 day ($p = 0.009$ for powder, $p = 0.02$ for aqueous extract and $p = 0.001$ for 80% ethanol extract). In addition to hyperglycemic effect *A. indica* leaf also showed hypolipidemic effect. Serum total Cholesterol and TG level decreased significantly by different extracts and powder of *A. indica.* Glibenclamide as expected, ameliorated diabetic condition significantly ($p<0.05$). Body weight of the rats tends to increase throughout the study period. No significant change was observed in serum insulin level. The obtained results suggest that *A. indica* leaf improves the glycemic and lipidemic status of type 2 diabetic rats. The efficacy of *A.indica* leaf (powder, aqueous extract and 80% ethanol extract) as hypoglycemic and anti-lipidemic agents was found ethanol extract > aqueous extract > powder.

**INTRODUCTION:** Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine disorder based disease. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus [1]. Though pharmaceutical drugs like sulfonylureas and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications [2, 3]. In recent years, there has been renewed interest in plant medicine [4, 5, 6] for the treatment against different diseases as herbal drugs are generally out of toxic effect [7, 8] reported from research work conducted on experimental model animal. Although in human, whether there is any toxic effect are not investigated. Isolated studies screened various plants having "folk medicine reputation" by biochemical test for this of antidiabetogenic effect [9].

*Azadirachta indica* (family- Meliaceae [Melioideae]), a medium-sized tree, is found throughout the South Asian region. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity.

Traditional healers claim that the leaves of the plant possess antidiabetic properties. Scientific reports also support the hypoglycemic activity of this plant [10, 11, 12, 13]. Although there exists a substantial number of reports claiming the hypo/anti-hyperglycemic effect of different parts (leaves, flowers, seeds, fruits, roots and bark) of this plant in normal as well as STZ (streptozotocin) induced diabetic animals, there was no published report regarding the comparative study of *A. indica* leaf crude powder and different extracts (aqueous and 80% ethanol) on glucose homeostasis. So the aim of this present study is to find out the comparative study of *A. indica* on glycemic, insulinemic and lipidemic status of type 2 diabetic model rats.

**MATERIALS AND METHODS**:

**Plant materials and preparation of test sample:** The fresh green leaves of *Azadirachta indica* Linn. were collected from Kushtia, Bangladesh. The collected fresh leaves of *Azadirachta indica* were weighed (3.630 kg), washed and air dried hygienically. The dried leaves were homogenized to powder with the help of grinder and 1.910 kg powder was obtained. Then the powder was sieved and 1.635 kg of fine powder was obtained which was stored in the refrigerator at -20$^{o}$C.

500g each of dried leaf powder were dissolved in 4.5L of boiled water and 1.5L of 80% ethanol. These suspensions were filtered with thin and clean cloth and then filtered by filter paper. The suspensions were then dried by BUCHI Rota vapor R-114, connected with BUCHI water bath B-480 at 40$^{o}$C. Finally the semi-dried extracts were dried using a freeze-drier (HETOSICC, Heto Lab Equipment Denmark) at -55$^{o}$C temperature and finally 85.60g and 65.30g of water and ethanol extracts were obtained respectively.

**Experimental Animals:** The study was conducted on adult Long-Evans (both sexes) rats (weighing 150-240g) bred at the BIRDEM animal house maintained at a constant room temperature of 22±5$^{o}$C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure when animals were used after 12hrs fasting. The rats had no access to food during the whole period of blood sampling. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

**Induction of Type 2 Diabetes to the Rats:** Diabetes stimulating Type 2 was induced by a single intra-peritoneal injection of streptozotocin (90mg/kg

body weight, dissolved in 0.1 citrate buffer, pH 4.5) to 48hr old pulps of Long-Evans rats as described by Bonner-Weir *et al*., 1981 [14]. Experiments were carried out 3 months latter to STZ injection and those rats having blood glucose level 8-12 mmol/l at fasting condition, were considered to carry out the experiments.

**Experimental design:** The experimental diabetic rats were divided into five groups as follows:

- Water control group: Rats of this group received deionized water at a dose of 10ml/kg bw.
- Positive control group: This group of rats received Glibenclamide at a dose of 5 mg /kg bw.
- Test group 1(Crude powder): *Azadirachta indica* crude powder was given with normal food at a dose of 1.25gm/kg bw. The normal food was contained wheat (42%), wheat bran (20%), fish meal (10%), oil cake (10%), gram (6%), pulses (5%), milk (4%), soyabean oil (1%), molasses (1%) and salt (1%). The crude powder was mixed with normal food and water was added to make a cake shape and given twice a day (9am and 3pm).
- Test group 2 (Water extract): Water extract was administered orally at a dose of 1.25gm/10ml water/kg bw.
- Test group 3 (ethanol extract): Ethanol extract was administered orally at a dose of 1.25 gm/10ml water/kg bw.

Rats of all groups were kept under similar environmental conditions, and were provided with enough food and water throughout the experiment. The body weight of each rat was measured in each week.

**Chronic study:** The ethanol extract (1.25 gm/10 ml/kg bw), water extract (1.25gm/10ml water/kg bw), crude powder (1.25gm/kg bw), glibenclamide (5 mg/10 ml/kg bw), and water (10 ml/kg body weight) were fed to the rats for 28 consecutive days. An oral glucose tolerance test (OGTT) was performed on the 0, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day of the study. Blood samples were collected to measure serum glucose, total cholesterol, triglyceride and serum insulin levels.

**Biochemical analysis:** Serum glucose was measured by glucose-oxidase method (Sera Pak, USA). The total cholesterol and triglyceride (TG) was measured by enzymatic- colorimetric method (Randox Laboratories Ltd., UK). Serum insulin by Rat Insulin enzyme linked immunosorbent assay (ELISA) method (Crystal Chem Inc., USA). The absorbance was measured by microplate ELISA Reader (Bio-Tek EL-340, USA).

**Statistical Analysis:** Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 12 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD or as Median (Range) as appropriate. Statistical analysis of the results were performed by using the student's t-test (paired and unpaired) or ANOVA (analysis of variance) followed by Bonferroni post Hoc test or Mann Whitney (u) test. The limit of significance was set at $p<0.05$.

**RESULTS:**

**Effect on the body weight (BW):** There was a tendency to increase the body weight in all groups. However, only aqueous extract on $7^{th}$ day showed a significant increase ($p = 0.04$) in comparison with ethanol extract treated group (**Table 1**).

**TABLE 1: CHRONIC EFFECT OF *AZADIRACHTA INDICA* (LEAF) ON BODY WEIGHT (GM) OF TYPE 2 DIABETIC MODEL RATS**

| Treatment | Experimental period (day) | | | | |
|---|---|---|---|---|---|
| | BW_0 (gm) | BW_7 (gm) | BW_14 (gm) | BW_21 (gm) | BW_28 (gm) |
| WC (n=7) | 177±22 | 187±21 | 197±19 | 201±21 | 209±22 |
| Gli (n=7) | 178±16 | 190±18 | 194±20 | 197±22 | 207±20 |
| Powd (n=7) | 175±20 | 183±20* | 182±26 | 192±29 | 195±28 |
| Aqu_E (n=7) | 201±27 | 207±26 | 216±24 | 217±24 | 228±26 |
| Eth_E (n=6) | 163±15 | 170±20 | 180±30 | 181±31 | 189±34 |

Results are expressed as Mean ± SD; *p<0.05; WC = Water control; Gli = Glibenclamide; Powd = Crude leaf powder; Aqu_E = Aqueous extract; Eth_Ext = Ethanol extract; Between group comparison was done using one way ANOVA with post Hoc Bonferroni test

**Chronic effect on glucose homeostasis:** Results of fasting serum glucose (FSG) level of the studied rats at baseline (before onset of feeding i.e., 0 day), at 7 day, 14 day, 21 and at 28 day of feeding are showed in **Fig. 1**. It was found that the crude powder of *A. indica* ameliorated diabetic condition of the model rats to a better extant than Glibenclamide [p=0.008 (7th day); p= 0.04 (14th day); p=0.001 (21st day) and p=0.009 (28th day)]. Aqueous extract of A. *indica* decreased serum glucose level significantly on 28th day [FSG mmol/l (M±SD) 8.38±1.57 on 0 day vs. 6.90± 1.07 on 28th; p=0.026]. Ethanol extract of *A. indica* lowered FSG significantly on 21st and 28th day (p=0.001).

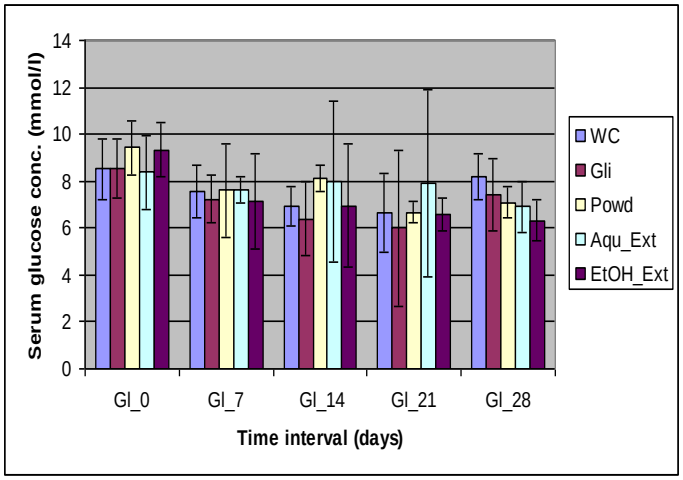


**FIG. 1: CHRONIC EFFECT OF *A. INDICA* (LEAF) ON FASTING SERUM GLUCOSE LEVEL OF TYPE 2 MODEL RATS**

**Effect on serum cholesterol:** It was found that in WC group, the level of serum cholesterol increased at the end of the study period. On the contrary, Glibenclamide significantly decreased total serum cholesterol after 28 days feeding (p=0.04). In case of crude powder, there was 3% increase in comparison to the 0 day value. By the influence of aqueous extract and ethanol extract of *A. indica* 13% and 6% reduction were noticed respectively after 28 days study. It is noteworthy that the decrease in the total cholesterol level by aqueous extract was significant (p= 0.02) (**Table 2**).

**TABLE 2: CHRONIC EFFECT OF *AZADIRACHTA INDICA* (LEAF) ON TOTAL SERUM CHOLESTEROL OF TYPE 2 DIABETIC MODEL RATS**

| Treatment | CHO_0 (mg/dl) | CHO_Final (mg/dl) |
|---|---|---|
| Water Control (n=7) | 65±6 | 69±13 |
| Glibenclamide (n=7) | 75±9 | 61±9* |
| Leaf Powder_ *A. indica* (n=7) | 52±6 | 54±7 |
| Aqueous_Ext_ *A. indica* (n=7) | 70±7 | 61±9* |
| EtOH_Ext_ *A. indica* (n=6) | 68±11 | 64±8 |

Data are presented as Mean±SD and compared using paired't' test. *p<0.05; `n' indicates number of rats in each group

**Effect on serum triglyceride (TG):** It was found that all of the groups had decreased tendency regarding to serum TG level in comparison to baseline value. The reducing tendency of TG level among different groups were 17%, 18%, 60% and 24% for WC,

powder, aqueous extract and ethanol extract respectively. While comparing between groups (using Mann-Whitney U test), aqueous extract showed the most significant triglyceride lowering effect ($p< 0.035$) (**Table 3**).

**TABLE 3: CHRONIC EFFECT OF *AZADIRACHTA INDICA* (LEAF) ON SERUM TRIGLYCERIDES (TG) LEVEL OF TYPE 2 DIABETIC MODEL RATS**

| Treatment | Serum Triglyceride level 'Median (Range)' | |
|---|---|---|
| | 0 day (mg/dl) | Final day (mg/dl) |
| WC (n=7) | 95 (71-154) | 79 (63-156) |
| Gli (n=7) | 78 (61-254) | 69 (49-159) |
| Powd_*A. indica* (n=7) | 97 (64-131) | 80 (68-107) |
| Aqu_Ext_ *A. indica* (n=7) | 150 (71-167) | 60 (44-76) |
| EtOH_Ext_ *A. indica* (n=6) | 117 (91-272) | 89 (73-124) |

Results are expressed as Median (Range); $p<0.05$; WC = Water control, Gli = Glibenclamide, Powd= Leaf powder, Aqu_Ext = Aqueous extract, EtOH_Ext = 80% ethanol extract, between group comparison was done using Mann -Whitney U test

**Effect on serum insulin level:** No significant change in serum insulin level was found in case of the test samples after 28 days period (**Table 4**).

**TABLE 4: CHRONIC EFFECT OF *A. INDICA* (LEAF) ON SERUM INSULIN LEVEL OF TYPE 2 DIABETIC RATS**

| Treatment | Serum Insulin level 'Median (Range)' | |
|---|---|---|
| | 0 day (ng/ml) | Final day (ng/ml) |
| WC (n=7) | 1.145 (0.978-1.532) | 0.693 (0.194-1.331) |
| Gli (n=7) | 1.035 (0.602-1.768) | 1.155 (0.682-2.499) |
| Powd_*A. indica* (n=7) | 1.335 (0.782-1.745) | 0.848 (0.378-1.332) |
| Aqu_Ext_ *A. indica* (n=7) | 1.253 (0.362-1.993) | 0.839 (0.362-1.806) |
| EtOH_Ext_ *A. indica* (n=6) | 0.927 (0.378-1.155) | 0.482 (0.105-0.682) |

Results are expressed as Median (Range); $p<0.05$; WC = Water control, Gli = Glibenclamide, Powd = Leaf powder, Aqu_Ext = Aqueous extract, EtOH_Ext = 80% ethanol extract, between group comparison was done using Mann -Whitney U test

**DISCUSSION:** Oral hypoglycemic agents and insulin is the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, however, they have prominent side effects and fail to significantly alter the cause of diabetic complications [15]. As the knowledge of heterogeneity of this disorder increases, it is needed to look for more efficacious agents with lesser side effects. Though the development of modern medicine results in the advent of modern pharmacotherapeutics (in additions to insulin, sulphonylureas and biguanides) like $\alpha$-glucosidase inhibitors and thiazolidinediones, there is still a need to look for new drugs as the existing drugs do not modify the course of diabetic complications. Therefore, as the disease is progressing unabated, there is an urgent need of identifying indigenous natural resources with antidiabetic properties in order to develop them as new therapeutics.

The present study was undertaken to assess the antidiabetic effect (chronic) with underlying mechanism of action of *Azadirachta indica* crude leaf powder, aqueous and ethanol (80%) extract on streptozotocin induced type 2 diabetic model rats. This study also compares the antidiabetic effect of different extracts of *A. indica* on Type 2 diabetic model rats.

In the present study *A. indica* leaf powder, aqueous extract and 80% ethanol extract was fed to type2 diabetes rats for 28 consecutive days. In our study, we found that the type 2 rats from all groups gained in body weight throughout the study period. The rise was not significant except aqueous extract on 7$^{th}$ day showed a significant increase ($p= 0.04$) in comparison with ethanol extract treated group. Our findings are in accordance with the findings of Hussain *et al*., [16] who also observed the gain in body weight of rats treated with aqueous extract of *A. indica* for 16 weeks. The probable mechanism for increased weight in Type 2 rats treated with *A. indica* is due to its ability to reduce hyperglycemia.

Regarding to hypoglycemic activity, it was found that continuous treatment with *A. indica* powder and extracts for 28 days caused a significant reduction in serum glucose level of Type 2 diabetic rats. The results are consistent with others [12, 13, 17, 18, 19, 20].

The extent of hypoglycemic effect varied considerably among different groups of extracts. Ethanol extract of *A. indica* lowered serum glucose level on 21st day and 28th day (p=0.001, while comparing within groups). Again while comparing between groups none but ethanol extract showed the most significant hypoglycemic effect on 28 day (p=0.04) in comparison to water control. Aqueous extract also reduced the serum glucose level significantly (p=0.02) but the effect was non significant while comparing between groups. Some spontaneous reduction in serum glucose level was noticed in water control group on 14th day and 21st day but the glucose level went back to near base line level on day 28.

The possible mechanism underlying the hypoglycemic activity of different extracts of *A. indica* may be potentiation of pancreatic secretion of insulin from β-cell coupled with extra pancreatic mechanisms like decreased glycogenolysis and enhanced glycogenesis by the liver; and enhanced transport of blood glucose to peripheral tissues. As it was seen from our experiment, serum insulin level did not change significantly, rather there was comparatively less serum insulin level than the base line level in *A. indica* treated groups.

There is another similar report claiming that *A. indica* does not modulate insulin effect on glycogen metabolism [21] rather it blocks the inhibitory effect of serotonin on insulin secretion/release in pancreas of rats mediated by glucose, eventually glucose control. In another report it was found that the increased peripheral glucose uptake was due to inhibitory action of insulin by inhibiting glycogenesis [20]. Hence, the obtained hypoglycemic effect by feeding *A. indica* for 28 days was probably, due to, increased peripheral utilization of glucose. *A. indica* leaves are known to have compound like flavonoids, quercetin which possess hypoglycemic activity. The presence of such constituent might be responsible for antidiabetic activity of *A. indica*.

Apart from the blood sugar lowering effect, beneficial changes in lipid profile have also been observed by *A. indica* extract and powder. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics. Since dyslipidemia plays an important role in the pathogenesis of macro- and microvascular complications of diabetes, hence, improvement in the lipid abnormalities must play beneficial role in inhibiting the complications of diabetes.

It has been claimed that hypercholesterolemia and hypertriglyceridaemia occurred in STZ induced diabetic rats [22]. Consecutive administration of *A. indica* powder and extracts for 28 days lowered serum triglycerides. Significant (p=0.035) reduction in triglyceride and serum cholesterol (P=0.02) level was observed with aqueous extract of *A. indica.* The obtained result is supported by the findings of other investigator [23]. These findings revealed that a continuous administration of the aqueous extract of *Azadirachta indica* improves serum lipids secondary to the diabetic state.

Thus, it can be concluded that, *Aadirachta indica* (leaf) has got promising antihyperglycemic and hypolipidemic effects in respect to Type 2 diabetic model rats and the accordance of its effect of efficacy can be explained as ethanol extract > aqueous extract > powder.

**ACKNOWLEDGEMENTS:** We gratefully acknowledge the financial support of International Program for the Chemical Sciences (IPICS), Uppsala, Sweden; International Foundation for Sciences (IFS), Stockholm, Sweden and Diabetic Association of Bangladesh in conducting this study.

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