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Phytochemical Screening and Effects of MethanolicExtract of*AzadirachtaIndica* Leaf in Alloxan Induced Diabetic Rats

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Abstract: *Phytochemical screening and antidiabetic effects of methanolic extract of Azadirachtaindica leaf was investigated in Alloxan induced diabetic rats. The phytochemical screening indicates the presence of alkaloids, flavonoids, saponins, steroids, tannins and phenolic compounds. The methanolic leaf extract of A. indica administered orally, significantly (P<0.05) lowered blood glucose concentration (mg/dl) in alloxan induced diabetic rats from (444.75 ± 36.36) to (83.00 ± 4.26) at 500mg/kg b.w while the standard drug Glibenclamide significantly (P<0.05) lowered blood glucose from (350.75 ± 60.91) to (84.50 ± 569) at 0.6mg/kg b.w. The methanolic leaf extract had no significant (P>0.05) effect on the weight of the rats. However, there was significant weight (g) loss for the untreated rats from (167.25 ± 2.01) to (129 ± 1.89). In this study, the results obtained revealed that A. indica is a potent hypoglycemic agent as Glibenclamide and could serve as a remedy in the management and treatment of diabetes mellitus.*

Keywords: *Hypoglycemia, diabetes, phytochemical, extracts*

I. Introduction

Diabetic mellitus is a metabolic disorder characterized by chronic hyperglycemia. It is a common and very prevalent disease affecting the citizens of both developed and developing countries. According to WHO statistics released on May 16, 2012, 10% of the world population is affected by this disease. Diabetic mellitus is caused by abnormality of carbohydrate metabolism which is linked to low insulin level or insensitivity of target organs to insulin. Maiti, *et al.,* (2004). Despite considerable progress in the treatment of diabetics by oral hypoglycemic agents, search for new drugs continues because the existing synthetic drugs have several limitations. Therapeutic efficacy of many plants has been described by many practitioners of herbal medicine .Antidiabetic medicinal plants were used to treat diabetics before the early 1920's (Chattopadhyau, *et al.,* 1999). Shapiro and Gong (2002) listed a number of plants to control blood glucose such as *Azadirachtaindica*and *Mormodicacharanti.* Previous studies had reported the beneficial effects of *A. indica* in the management of diabetes mellitus and amelioration of the oxidative stress associated with the disease (Shapiro and Gong, 2002 and Gupta, *et al.,* 2004). The present study was undertaken to determine the efficacy of the methanolic extract of *A. .indica*leaf as an antidiabetic agent in Alloxan induced rats and to compare its efficacy with the standard drug glibenclamide.

II. Materials and Methods

The research was carried out in Biochemistry laboratory of the Federal University of Technology, Minna, Nigeria between July and September, 2012.

Animals

Male and female wistar rats weighing between 143g to 180g were purchased from Nigeria Institute of Veterinary Research(NIVR) Jos, Plateaus State, Nigeria. They were housed in plastic cages under 36 – 37^{o}C temperature, 50% humidity and 12 hour light – dark cycle. The rats were fed with standard pellet and water ad libitum for two weeks to acclimatize prior to the experimentation.The animals were sacrificed in accordance with the laid down rules and regulations.

Chemicals

Alloxan monohydrate was purchased from BDH chemicals. The brand name of the standard Daoni (R) was Glibenclamide. Methanol and other reagents used were of analytical grade.

Plant Collection and Extract Preparation

Fresh leaves of *Azadirachtaindica*leaf were collected within the Bosso campus of the Federal University of Technology, Minna. The leaves were later confirmed by the Department of Biological Sciences, Federal University of Technology, Minna. The leaves were air dried for two weeks at room temperature before grinding to powdered form by an electric blender. 50g of the powdered leaves was transferred into 400ml of methanol, stirred to an homogenous mixture and then refluxed for 3 hours at 65^{o}C using soxhlet extractor (medline). The mixture was filtered using a Muslin cloth and subsequently with filter paper to obtain a fine filtrate. The filtrate was evaporated using a water-bath at 65^{o}C for few hours to obtain the extract yield. The extract yield was kept in a sterile bottle and refrigerated until. Prior to use, 1g of the extract was dissolved in 10ml of dimethylsulfoxide (DMSO).

Induction of Diabetes

0.5g of Alloxan was dissolved in 10ml of distilled water. A single intraperitoneal dose of 100mg/kg b.w. of Alloxanwas administered to the rats after fasted for 12 hours. Two hours after induction, the rats were fed. After one week of induction period, the rats with blood glucose level of 220mg/dl and above were considered diabetic and selected for the study.

Experimental Design

A total of sixteen (16) rats were used in the study (4-normal and 12 Alloxan induced diabetic rats). The animals were divided into four groups of (4 of 4) rats each. Group A comprises of the non induced animals, group B were induced but untreated, Group C were animals treated with 500mg/kg b.w. of methanolic leaf extract of *A. indica*and Group D were treated with standard drug Glibenclamide. (0.6mg/kg bw). (Satnishsekar*et al.,* 2005 and Abraira*et al.,* 2005). The treatment was done on a daily bases for twenty one days. The blood samples were collected from the tail vein of the animals to determine blood glucose level using a glucometer.

Estimation of Body Weight

The weight of all the rats were taken prior to induction of diabetes using an electronic weighing balance.

Phytochemial screening

The methanolic leaf extract of *Azadirachtaindica*was subject to phytochemical analysis to determine the presence of alkaloids, flavonoids, saponins, steroids, tannins and phenolic compound according to the method described by Talukdar*et al;* 2010.

Alkaloids

2ml of methanolic extract of *A. indica*was measured into a test-tube to which few drops of picric acid solution was added. An orange colouration was observed which indicate the presence of alkaloids.

Flavonoids

4ml of methanolic extract of *A. indica*solution was treated with 1.5ml of 50% methanol. The solution was warmed and metal magnesium was added. 5 drops of concentration HCl was added and a red colour was observed indicating presence of flavonoids.

Saponins

Saponins were detected by the froth test, 1g of the methanolic extract of *A. indica*leaf was weighed into a conical flask to which 10ml of distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for 30 seconds. It was allowed to stand for half an hour, formation of honey comb froth indicate the presence of saponins.

Steroids

Five drops of concentrated H_2SO_4 were added to 2ml of the methanolic extract of *A. indica*. A reddish brown colouration shows the presence of steroids.

Tannins

A little amount of methanolic leaf extract of *A. indica*was diluted with water, 3 drops of 10% ferric chloride solution was added. A blue color is observed for garlic tannins and green colour indicated catecholic tannins.

Phenolic Compounds

1ml of the methanolic leaf extract of *A. indica*was added to 1ml of 10% $FeCl_2$ and mixed together. The formation of a blue precipitate was observed which confirms the presence of phenols.

Statistical Analysis

Statistical analysis of the data was carried out by one way ANOVA.

III. Results

Table 1: Phytochemical constituents of methanolic leaf extract of *Azadirachtaindica*

Compound	Inference
Alkaloids	+
Flavonoids	+++
Saponins	+
Saponins	+
Steroids	+
Tannins	++
Phenolic compounds	++
Resins	-

+++ Heavily present
++ Moderately present
+ Present
- Undetected

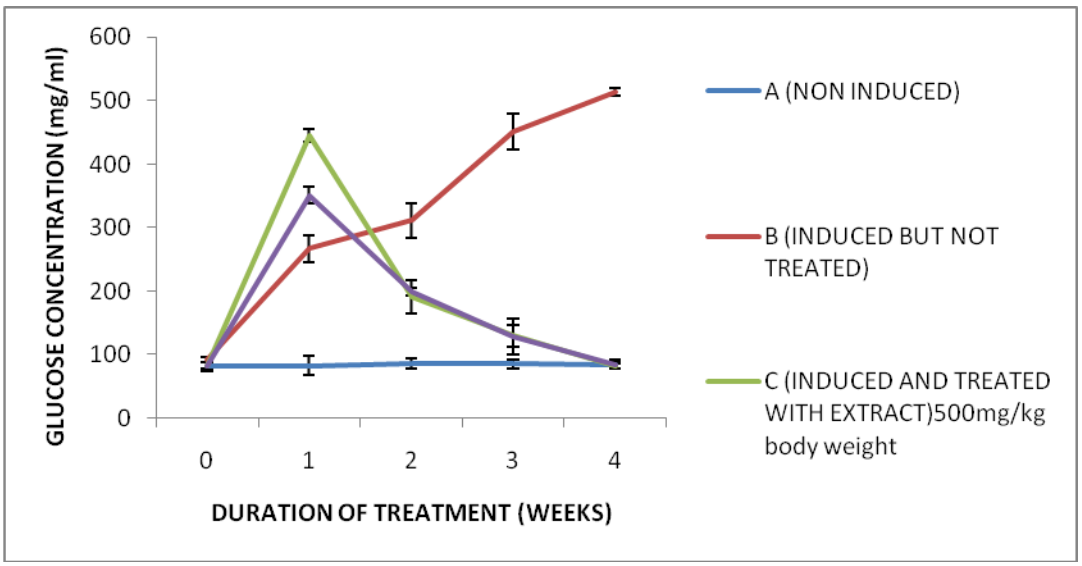

Fig. **1: Blood glucose concentration of non-induced rats, induced untreated rats, induced rats treated with *Azadirachtaindica*leaf extract, and induced rats treated with glibenclamide**

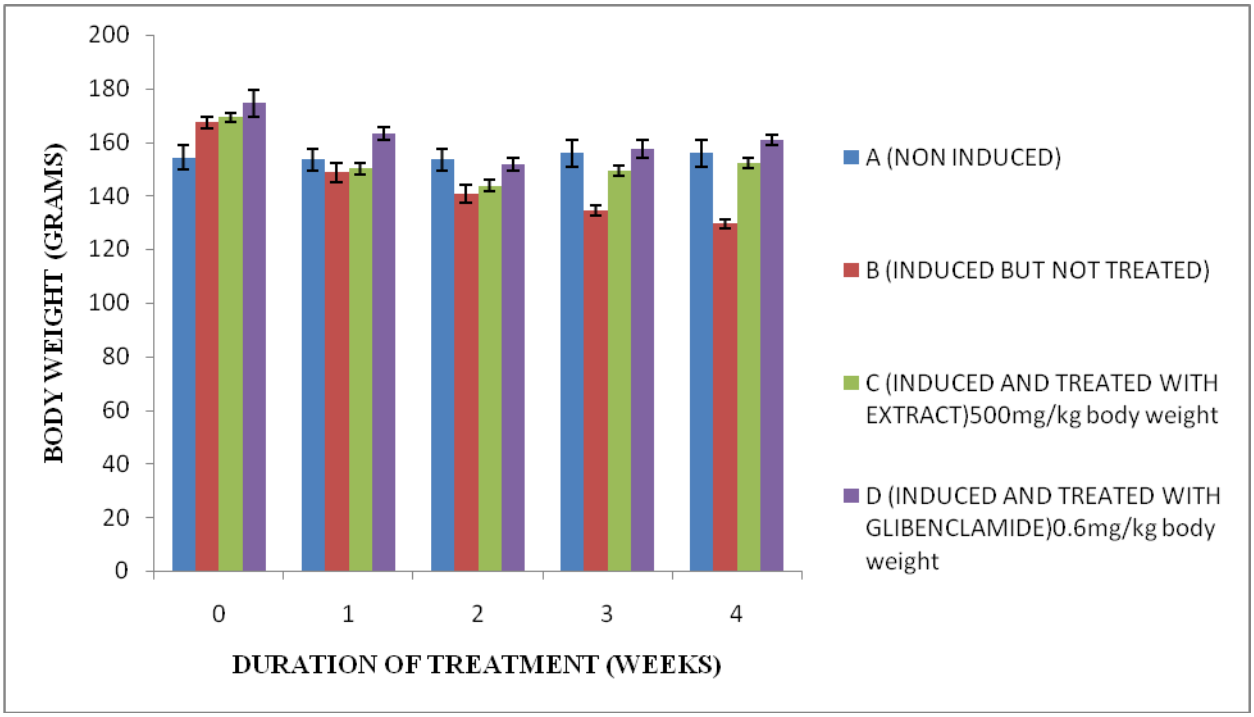

Fig. 2: Variation in body weight of non-induced rats, induced untreated rats, induced rats treated with *Azadirachtaindica*leaf extract, and induced rats treated with glibenclamide

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IV. Discussion

Medicinal plants have been used over the years as traditional alternatives in the management of diseases such as diabetes mellitus. The management of diabetes mellitus is achieved through effective regulation of blood glucose concentration which is a key step in preventing or reversing the complication associated with both Type 1 and 2 patients (Abraira*etal*, 1995). The phytochemical screening as depicted in Table 1 clearly indicates the presence of several phytoconstituents namely flavonoids, tannins, phenolic compounds, alkaloids, saponins and steroids. The results further revealed the complete absence of resins. Phytoconstituents such as flavonoids and phenolic derivatives mainly are known to boost the insulin secretion and scavenge free radicals in diabetic condition (Markes and Farnsworth, 1995). Flavonoids are also known to regenerate the damaged beta cells in diabetic rat which in turn elevate the insulin levels while phenolic compounds are effective antihyperglecemic agents (Oyedemi*etal*, 2011). This findings are in agreement with similar results obtained by other researchers e.gmethanolic extract activity of *Cajanuscajan*(Adaobi*etal.*, 2010). The findings of Shain*etal.*, 2009 also corroborated that most hypoglycemic plants extract contain phytochemicals as found in the case of aqueous leaf extract activity of *Trichusanthesdioica*. In the present study, a single dose administration of methanolic extract of *A. indica* effectively lowered the blood glucose level in induced diabetic rats compared to their control (Fig. 1). Alloxan partially damages selectively the pancreatic insulin secreting beta cells, leaving less active cells and resulting in a diabetic state (Junod*etal.*, 1969). Thus, the extract may act by a direct stimulation of insulin secretion in remaining beta cells. This effect may be attributed to compounds like (-) – epicateclin (Swarnalakshin*etal.*, 1981), Kampferol, quercetolrhamnosides (Arya*etal.*, 1989) andβ-sitosterol 3 – beta – D glucoside (Ivorra*etal.*, 1988) known to be present in most plants and reported to be insulin secretagogues (Sheehan and Zemantis, 1983). On the other hand, the action of the extract may be through insulin like extrapancreatic mechanisms such as the stimulation of glucose utilization and the reduction of hepatic gluconeogenesis (Ali *etal.*, 1993 and Gray *etal.*, 2000). The findings of this research corresponds with

other revelations like the effect of aqueous leaf extract of *A. indica* by Rahman*etal,* 2005 and chloroform leaf extract activity by Menakshi*et al.,* 2011 which had similar effects on diabetic rats. The methanolic extract of *A. indica*as presented in Fig. 2 indicates a non significant ($P > 0.05$) weight loss in non-induced rats which corresponds with the findings of Mishra and Singh 2005. However, this results does not correspond with the effect of aqueous leaf extract of *A. indica*on body weight of treated normoglyemic rats (Rahman*etal.*,2005). Statistical analysis of the study reveals no significant ($P > 0.05$) difference between blood glucose concentration of rats treated with methanolic extract of *A. indica*leaf and glibenclamide. This study indicates that methanolic extract of *A. indica*leaf is a potent antidiabetic agent comparable to glibenclamide activity and may be recommended as a remedy in the management of diabetes mellitus.

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