Original Research Article

Comparative anti-diabetic effects of the ethanol leaf-extracts of *Vernonia amygdalina* and *Azadirachta indica* in albino rats

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A B S T R A C T

K e y w o r d s Anti-diabetic effect, *Vernonia amygdalina*, *Azadirachta indica*, Albino rats	The comparative anti-diabetic effects of ethanol leaf-extracts of *Vernonia amygdalina* and *Azadirachta indica* were conducted. The animals were grouped according to the doses of extracts administered (0mg/kg, 200mg/kg, 400mg/kg and 600mg/kg body weight). The anti-diabetic effect was studied with a glucometer after a single administration of alloxan at 100mg/kg body weight. The administration by oral intubation of the extracts of *Azadirachta indica* at 200mg/kg, 400mg/kg and 600mg/kg body weight lowered the glucose levels significantly (P < 0.05) by 16.13%, 26.85% and 31.26% respectively; the extract of *Vernonia amygdalina* gave a corresponding percentage reductions of 54.71%, 56.62% and 57.93%, while the control group had an increase of 33.33%. There were significant dose-dependent reductions in the body weights of all the animals administered the extracts. The results showed that the extract of *Vernonia amygdalina* was more effective as an anti-diabetic agent than *Azadirachta indica* extract.

Introduction

The use of medicinal plants started from the time of our ancestors in their attempt to treat diseases and relieve physical sufferings. According to legend, the first man to practice the art of healing using medicinal plant in the Yoruba speaking part of Nigeria was Orunmua (Abayomi, 1993). These methods of using plants medicinally must have come to the early man in the most unscientific way. Since then, the knowledge of medicinal plant has continued to be useful in the production of drugs, food, spice, perfume and preparation of surgical dressings (Abayomi, 1993). The retention of medicinal practices and traditions is evident mainly in the rural communities. With modern technology, medicine has moved from a purely traditional phase to high technological production of synthetic chemical and the extraction of chemicals from plants to produce drugs. Several of these drugs are derived from plants that form the basis of traditional medicine (Bergman, 2000). The research of plants' bioactive substances has contributed immensely for the betterment of mankind through the provision of value added economic returns, manufacturing of natural

plant production industry, provision of better health care and increase in export earning in both rural and urban areas (Tona, 2002).

Azadirachta indica (Neem) and *Vernonia amygdalina* (Bitter leaf) are well known as versatile medicinal plants having a wide spectrum of biological activities both nutritionally and pharmacologically. Bitter leaf, after squeezing and soaking in several changes of water can be used for soup, and served with garri especially in south eastern zone of Nigeria. Bitter leaf is also eaten by Creoles (ethnic group) of Sierraleone, who specially cultivate it (Yumah, 1991). The leaves are sometimes rubbed on the body for itching, parasitic skin diseases, ring worm etc. The leaves in food preparation act as digestive, tonic and antiscorbutic agents (Burkill, 1985).

Plants continue to be a major source of medicine, as they have been throughout human history. Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. Traditional medicine is an act of using plant leaves, barks, roots or any other part in the crude form for the treatment of various diseases (Ebe, 2004). In some cases, plant leaves are eaten raw, squeezed or parboiled to extract the active ingredients contained in the plant parts. In the pre-christian era, traditional medicine was virtually the only means of curing infection (Ugwu *et al.*, 2005).

Azadirachta indica (neem) is a tree in the mahogany family. It is one of the species in the genus "*Azadirachta*" and is native to India, Bangladesh and Pakistan, growing in tropical and semi-tropical regions. Other names include dogonyaro, vimba and kohomba. In East Africa, it is also known as muarubaini which means the tree of the 40, as it is said to treat 40 different diseases

(Ganguli, 2003). It is an evergreen tree, cultivated in various parts of the world. Every part of the tree has been used as traditional medicine for house hold remedy against various human ailments from antiquity (Koul *et al.*, 1990). Neem has been extensively used in ayurveda and homeopathic medicine and has become a cynosure of modern medicine. The importance of the neem tree has been recognized by the US national Academy of Sciences, which published a report in 1992 entitled "Neem – a tree for solving global problems". The advancement of neem research has been documented (Schmutterer, 1995; Singh *et al.*, 1996).

Vernonia amygdalina (Bitter leaf) is one of the widely studied medicinal plants. It belongs to the family "compositae". It occurs as a small shrub with height from 2-5m, the stem is rough with young branches and petiolate green leaves of about 6mm diameter. The leaves are elliptic in shape, short acuminate at the apex and slight chordate at the base. The bitter taste of the plant leaves gives rise to its common name "Bitter Leaf" (Nwobegu and Egbuna, 2002). The bitterness can be abated by boiling or soaking in several changes of clean water (Mbinglo, 1998). It is used in Nigerian folk medicine as a tonic and remedy against constipation, fever, high blood pressure and many infectious diseases. The stem and root divested of the bark are used as chew-sticks in Nigeria (Taiwo *et al.*, 1999). All parts of the plant are pharmacologically useful. The roots and leaves are used in the treatment of fever, malaria, diarrhea, dysentery, kidney problems, and stomach discomfort among several other uses (Iwalokun *et al.*, 2006).

This work was aimed at conducting further studies on both *Vernonia amygdalina* and *Azadirachta indica* leaves to elucidate their comparative antidiabetic properties.

Materials and Methods

All the chemicals and reagents were of analytical standard. The samples (*Azadirachta indica* and *Vernonia amygdalina* leaves) were obtained from Abakaliki in Ebonyi State, while the animals (albino rats) were gotten from University of Nigeria, Nsukka in Enugu State.

Extraction of Plant Materials

500g each of dry and ground *Vernonia amygdalina* and *Azadirachta indica* leaves was soaked in 1000mls of ethanol in a container such that the ethanol would cover the sample. It was left standing for about 48 hours. The solution was squeezed and filtered with a muslin cloth, and the filtrate was poured into an evaporation dish. It was then exposed to air and mild heat of the sun until a semi-solid extract was gotten.

Animal Grouping for Alloxan Administration

Rats were grouped into eight (AA, BA, CA, AV, BV, CV, D and E) containing four animals in each group and alloxan was intraperitonially administered into all the animals except those in group E (control) at the dose of 100mg/kg body weight.

Determination of Glucose Levels after Alloxan Administration

Blood samples from the rats were collected through the tail vein and tested for hyperglycemia with a glucometer after three days.

Administration of Extracts after Alloxan Administration

Within a period of fourteen days, the animals in groups AA, BA, and CA were administered the ethanol extract of *Azadirachta indica* at 200, 400, and 600mg/kg body weight respectively with corresponding administration of the ethanol extract of *Vernonia amygdalina* to the animals in groups AV, BV, and CV while those in group D received 0mg/kg body weight of the extracts. The administrations were by oral intubation.

Determination of Glucose Levels within Fourteen Days of Administration of Extracts

Blood samples from the rats were collected through the tail veins and tested for glucose levels on daily basis within the fourteen days of administration of extracts using a glucometer.

Determination of Body Weights of Diabetic Animals within Fourteen Days of Administration of Extracts

The body weights of the animals were measured on daily basis with a weighing balance.

Data Analysis

All the tested parameters were subjected to statistical analysis using T-test. Differences between means were regarded significant at $P<0.05$ (Oyeka, 1996).

Results and Discussion

There were significant elevations in the mean plasma glucose levels of all the animals administered alloxan after three days (Table 1). The elevated mean plasma glucose levels might have resulted from the effect of alloxan on the pancreatic β - cells that give rise to impaired insulin secretion that translates into low plasma glucose utilization (Wohaieb, 1987; Weiss and

Sumpio, 2006). Three days after the administration of alloxan, a decrease in the physical activities of the animals was observed. Inability of animals to use glucose as a source of energy results in asthenia (Prenkti, 1997). An increase in water intake was observed. This could be due to alloxan induced hyperosmorality resulting in depletion of intracellular water, triggering the osmoreceptors of the thirst center of the brain (Kruzynska and Olefsky, 1996). Alloxanised animals display a continuous decrease in body weights (Kamanayake *et al.*, 1990). Chattopadhyay and Maitra (2003) noticed that alloxanized animals exhibited a low physical activity, loss in body weights and increase in water intake. The decrease in weights and increase in water intake owe to the ability of alloxan to dehydrate the body by hyperosmolarity resulting in depletion of intracellular water and stimulating the osmoreceptors of the thirst center of the brain (Genuth, 2006).

Both extracts significantly reduced glucose levels dose-dependently while *Vernonia amygdalina* exerted more hypoglycemic effect (Fig. 1 & 2). Oral doses of neem leaf extracts reduced insulin requirements between 30% and 50% for non-insulin dependent disease, and insulin-sensitive diabetes (Atawodi, 2006).

Both extracts of the plants induced some significant gain in body weights (Fig. 4 & 5). This could be a sign of recovery from diabetes possibly because of the administration of the extracts. *Vernonia amygdalina* seemed to be more effective.

The extract of *Vernonia amygdalina* exhibited more positive anti-diabetic effect than that of *Azadirachta indica.* The extracts may be beneficial in the management of diabetes mellitus.

Table.1 Mean Plasma Glucose Concentrations (mg/dl) Three Days after the Administration of Alloxan.

ANIMAL GROUP	MEAN PLASMA GLUCOSE CONCENTRATIONS (mg/dl)
AA	114.10 ± 2.10
BA	115.10 ± 1.90
CA	114.20 ± 1.50
AV	141.30 ± 3.40
BV	126.80 ± 2.10
CV	120.50 ± 3.30
D	160.50 ± 3.20
E	76.86 ± 4.64

There were significant ($p<0.05$) elevations of glucose concentrations in all the animals administered alloxan. It gave a clear indication that the alloxan was effective.

Keys

AA = *Azadirachta indica* group administered 100mg/kg body weight of alloxan
BA = *Azadirachta indica* group administered 100mg/kg body weight of alloxan
CA = *Azadirachta indica* group administered 100mg/kg body weight of alloxan
AV = *Vernonia amygdalina* group administered 100mg/kg body weight of alloxan
BV = *Vernonia amygdalina* group administered 100mg/kg body weight of alloxan
CV = *Vernonia amygdalina* group administered 100mg/kg body weight of alloxan
D = Animal group administered 100mg/kg body weight of alloxan (Alloxan control)
E = Animal group that received 0mg/kg body weight of alloxan (Control)

Fig.1 Plasma Glucose Concentrations of Diabetic Animals that Received
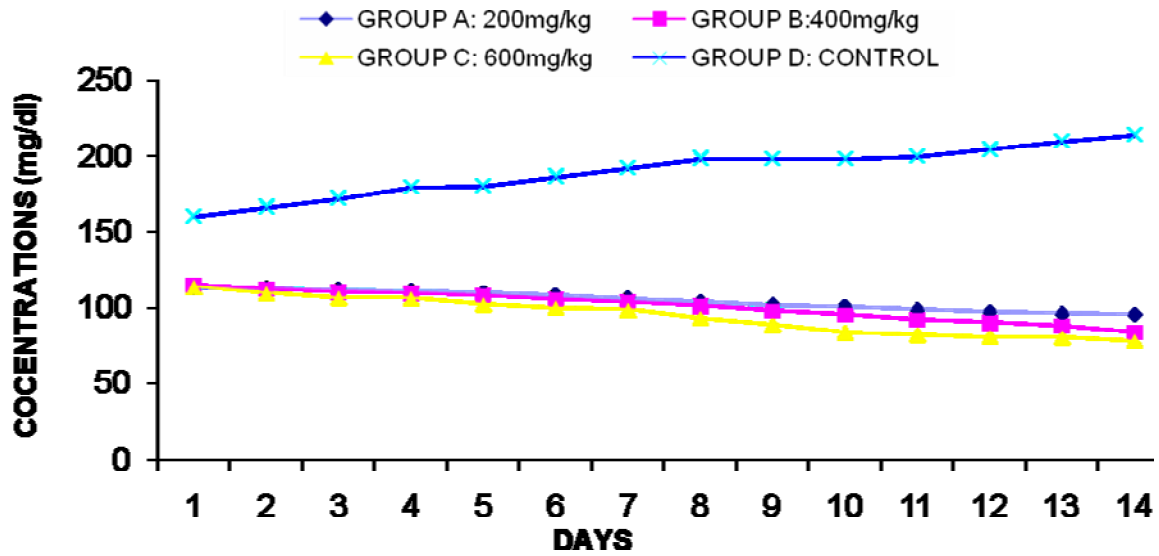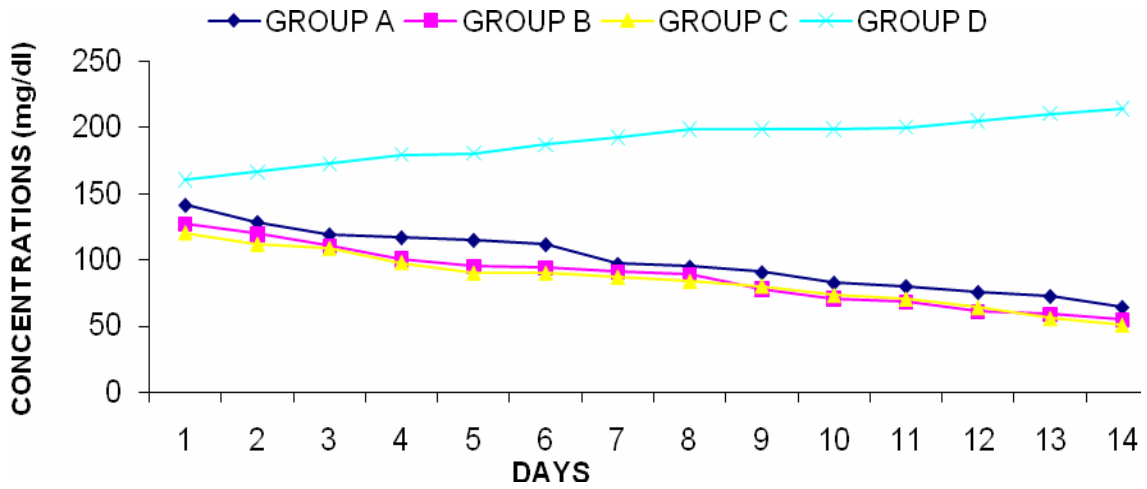*Azadirachta indica* extract within Fourteen Days

Fig.2 Plasma Glucose Concentrations of Diabetic Animals that Received Vernonia
amygdalina extract within Fourteen Days

Keys
A=200mg/kg
B=400mg/kg
C=600mg/kg
D = CONTROL

Fig.3 Percentage Change in Mean Plasma Glucose Concentrations for Diabetic Animals that Received *Azadirachta indica* and *Vernonia amygdalina* Extracts
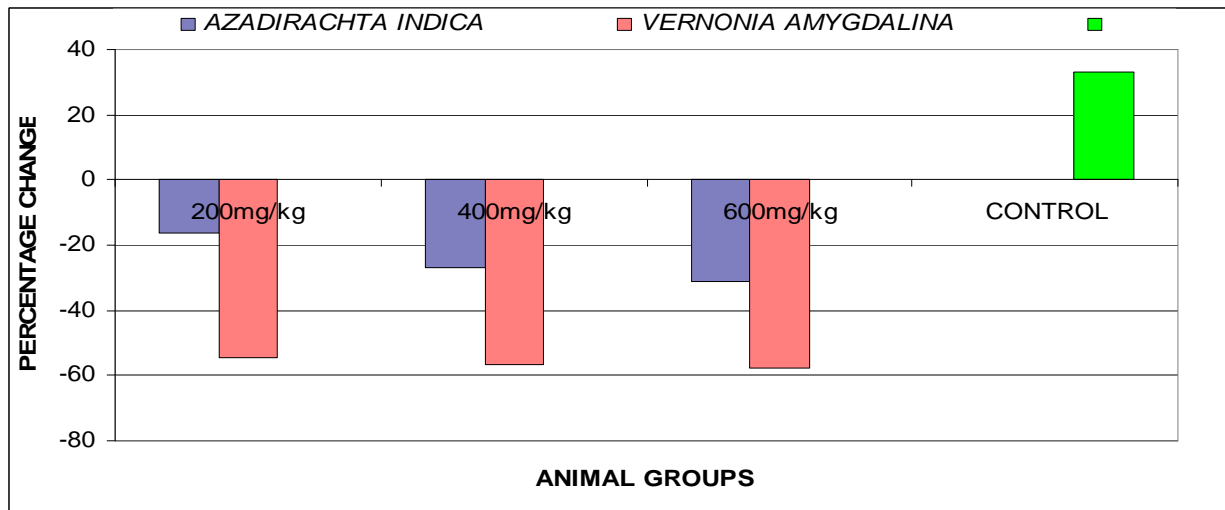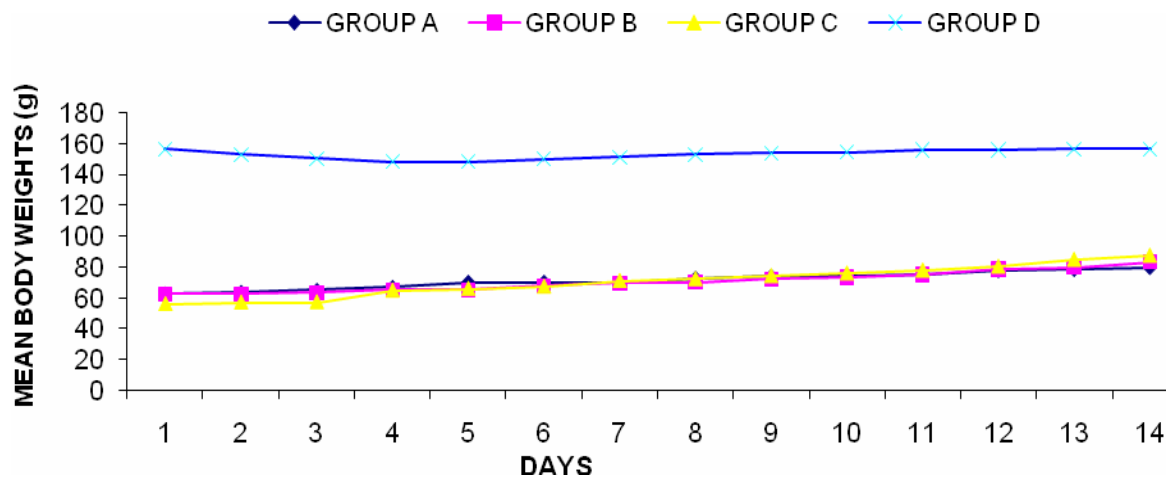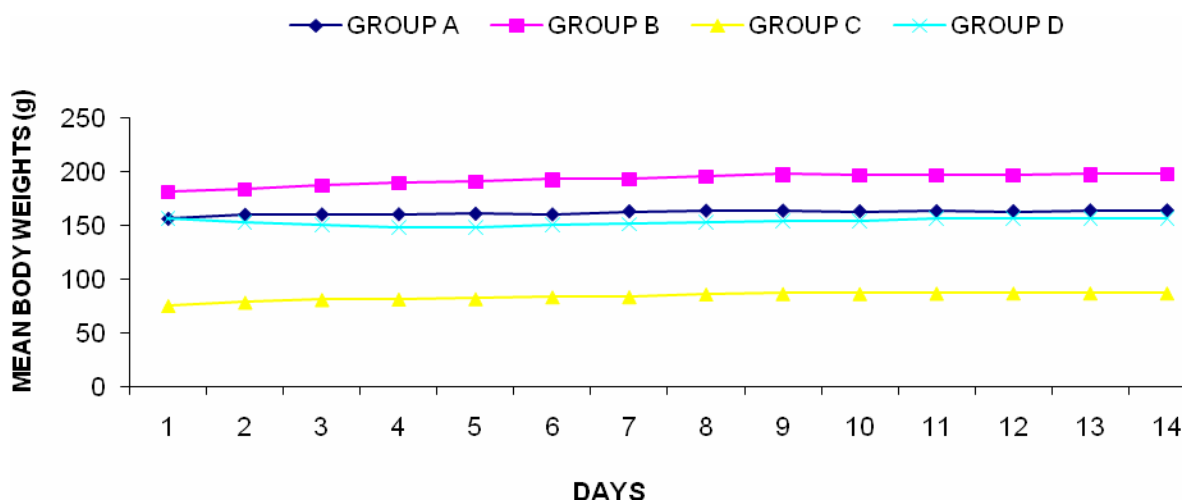

Fig.4 Mean Body Weights (g) of the Diabetic Animals given *Azadirachta indica* Extract.

Keys
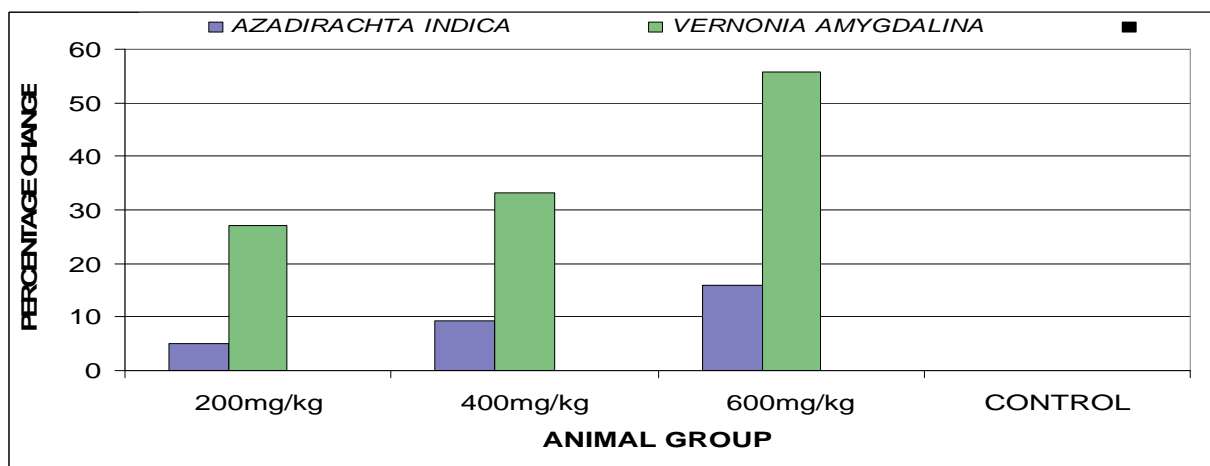A=200mg/kg
B=400mg/kg
C=600mg/kg
D = CONTROL

Fig.5 Mean Body Weights (g) of the Diabetic Animals given *Vernonia amygdalina* Extract.
There was a dose-dependent gain in body weights

Keys
A=200mg/kg
B=400mg/kg
C=600mg/kg
D = CONTROL

Fig.6 Percentage Change in Mean Body Weights of Diabetic Animals that Received
Azadirachta indica and Vernonia amygdalina Extracts

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