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Research Article

EFFECTS OF ETHANOL LEAF-EXTRACTS OF AZADIRACHTA INDICA LINN. (MELIACEAE) AND VERNONIA AMYGDALINA WIL. (ASTERACEAE) ON ALKALINE PHOSPHATASE, GAMMA-GLUTAMYL TRANSFERASE AND ALPHA-AMYLASE ACTIVITIES IN ALBINO RATS

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Abstract

The effects of ethanol leaf-extracts of Azadirachta indica and Vernonia amygdalina on alkaline phosphatase, alpha-amylase and gamma-glutamyl transferase activities were investigated using Wister albino rats through Spectrophotometric method. Fifty-six albino rats were grouped into seven (A, B, C, D, E, F and G) containing eight rats per group. Ethanol extract of Azadirachta indica leaves was administered to the animals in groups A, B and C at the dosage of 200 mg / kg, 400 mg / kg and 600 mg / kg , body weights respectively following oral route, while ethanol extract of Vernonia amygdalina leaves was administered to the animals in groups D, E and F at the dosage of 200 mg / kg, 400 mg / kg and 600 mg / kg , body weights respectively (orally), whereas group G (control) received no dose of the extracts. The administrations done through oral intubation were for fourteen days. Blood samples were collected through heart puncture on the fifteenth day following the last day of administration of the extracts. Both extracts exerted significant dose-dependent ($p \leq 0.05$) elevations in alkaline phosphatase and gamma-glutamyl transferase activities and corresponding significant reductions in alpha-amylase activities ($p \leq 0.05$). There were also significant dose- dependent reductions in the body weights of all the animals administered the extracts. The ethanol leaf-extract of Azadirachta indica exerted more elevation in the activities of alkaline phosphatase and gamma-glutamyl transferase than the extract of Vernonia amygdalina. Reductions in body weights and alpha-amylase activities were more in the groups treated with the extract of Azadirachta indica. Ethanol leaf-extract of Vernonia amygdalina therefore could be less harmful than the extract of Azadirachta indica.

Keywords: Ethanol leaf-extract, Azadirachta indica, Vernonia amygdalina, alkaline phosphatase, gamma-glutamyl transferase, albino rats.

Introduction

Traditional medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine [1]. It involves the use of natural things (mostly plants) to cure various diseases. There are no synthetic or artificial additives in traditional drugs. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [1]. Medicinal plants are plants which one or more of their parts contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [2].

Azadirachta indica (Neem) is a tree in the mahogany family, Meliaceae. It is one of the two species in the genus Azadirachta, and is native to India, Burma, Bangladesh, Srilanka, Malaysia, Pakistan and also in Africa, particularly in Nigeria [3]. It is evergreen, but in severe drought, it may shed most or nearly all of its leaves. The branches are wide spread. All parts of the plant such as leaves, bark, flower, fruit seed and roots have advantages in medical treatment and industrial products. Its leaves can be used as a drug for diabetes, eczema and fever [4].

Vernonia amygdalina, a member of the Asteraceae family, is a small shrub that grows in the tropical Africa. Vernonia amygdalina is commonly called bitter-leaf in English because of its bitter taste, Shiwaka in Hausa, Onugbu in Igbo and Ewuro in Yoruba. It occurs as a small tree with height from 2-5m [5]. It has a characteristic bitter taste due to high level of vernonine [5].

Alkaline phosphatase (ALP) comprises a group of enzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate [6]. It is found in the liver, bone, placenta, intestine and kidney. Alkaline phosphatase is needed in small amounts and has optimum pH of 10 which varies with the nature and concentration of the substances that are acted upon [6]. The measurement of alkaline phosphatase is used in the investigation of possible hepatobiliary and bone diseases [7]. When the cells are destroyed in the tissues, more of the enzymes leak into the blood and levels rise in proportion to the severity of the condition [8].

Gamma glutamyl transferase (GGT) is an enzyme that catalyses the transfer of gamma glutamyl from gamma glutamyl compounds to amino acid and peptide acceptors which is known to be localized in

the membrane of many epithelial cells. It is a membrane bound enzyme that occurs in many parenchymatous organs, but appreciably its activity is only found in the kidney, pancreas, liver, spleen and small intestine. The serum level of this enzyme is almost invariably determined by liver and bile duct disorder [9]. GGT activity rises in cholestic liver disease, hepatoma, liver metastasis, chronic hepatitis, alcoholic hepatitis, extra-hepatic obstruction etc. It is an important marker in detecting alcohol induced liver disease [10].

Alpha-amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva where it begins the chemical process of digestion. The catalytic activity of amylase in serum and urine increases in pancreatic diseases especially in the acute stage of the diseases and decreases in duodenal juice. Amylase tests are sometimes used to monitor treatment of cancers involving the pancreas and after the removal of gallstone that has caused gall bladder attacks [11]. With the understanding of the nature of amylase enzymes and their hydrolytic potentiality, the use of amylase enzymes has been extended to various fields such as brewing, textiles and detergent industries and they are the most important of carbohydrate degrading enzymes produced by microorganisms, animals and plants [12].

Many medicinal plants have been proven to adversely affect liver, heart, kidney and serum functions; and others are known to inhibit pathways leading to the synthesis of important cellular components. This research was aimed at studying the effects of ethanol leaf-extracts of Azadirachta indica and Vernonia amygdalina on alkaline phosphatase, gamma-glutamyl transferase and amylase activities using albino rats.

Materials

The leaves of Azadirachta indica and Vernonia amygdalina were collected from Ijebu-Ode, Oyo State, Nigeria, in the month of January while the albino rats were obtained from the Department of Pharmacology Animal house, University of Ibadan, Nigeria.

Methods

Extraction of Plant Materials

Azadirachta indica and Vernonia amygdalina leaves were each collected and dried under room temperature for fifteen days. The dried leaves were separately ground into powdery form. About 900g of each ground sample was soaked in 2 liters of ethanol for 48 hours. The solutions of the extracts were gotten after several squeezing and filtrations with muslin cloths. The solutions were also separately exposed to air and mild heat of the sun for 24 hours until semi-solid residues free of ethanol were obtained.

Administration of the Extract

The animals were divided into seven groups (A, B, C, D, E, F and G), having eight rats in each group. Animals in groups A, B and C were administered the ethanol extract of Azadirachta indica leaves at 200mg/kg, 400mg/kg and 600mg/kg body weights respectively while those in groups D, E and F received the ethanol leaf-extract of Vernonia amygdalina at 200mg/kg, 400mg/kg and 600mg/kg body weights respectively and animals in group G (Control) received no extract. The administration lasted for 14 consecutive days through oral intubation while food and water were given ad libitum.

Collection of Blood Samples

The blood samples were collected through heart puncture.

Determination of Serum GGT, α-Amylase and ALP Activities

The GGT, ALP and α-amylase activities were determined by standard methods of Teitz [13], Englehardt [14] and Rauscher et al. [15] respectively.

Statistical Analysis

All the tested parameters were subjected to statistical analysis using T-test. Differences between means were regarded significant at $P<0.05$ [16].

Results

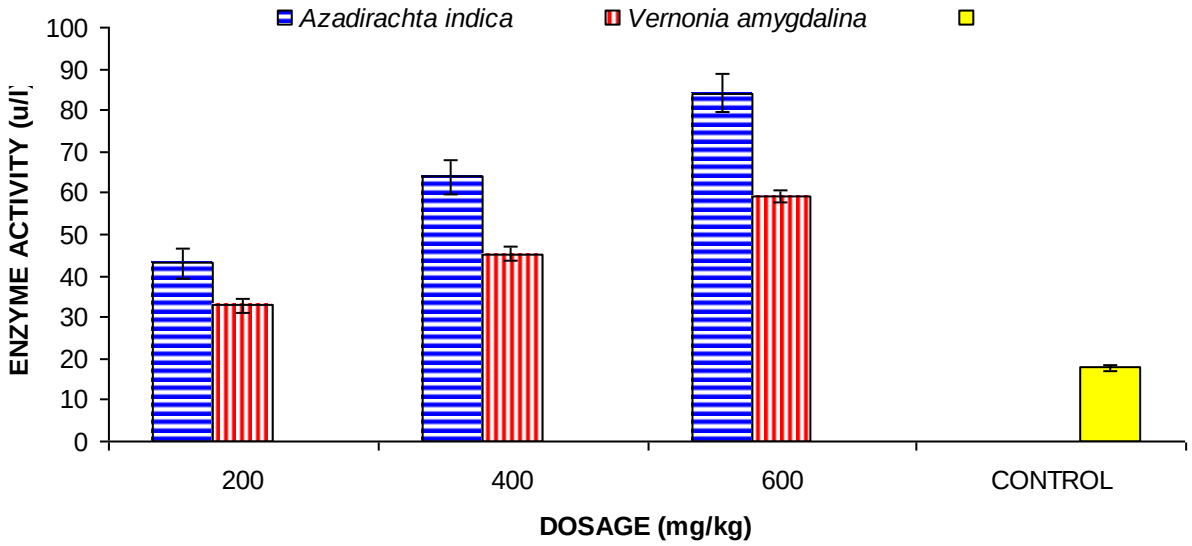

Fig. 1: Alkaline phosphatase activities (u/l) in the animals given leaf-extracts of Azadirachta indica and Vernonia amygdalina.

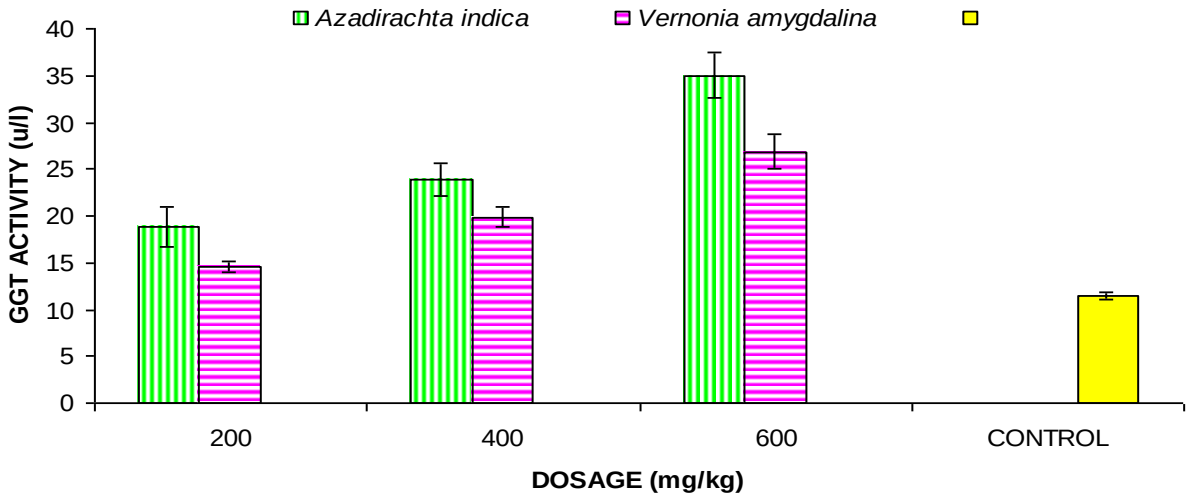

Fig. 2: GGT activities (u/l) in the animals that received leaf-extracts of Azadirachta indica and Vernonia amygdalina.

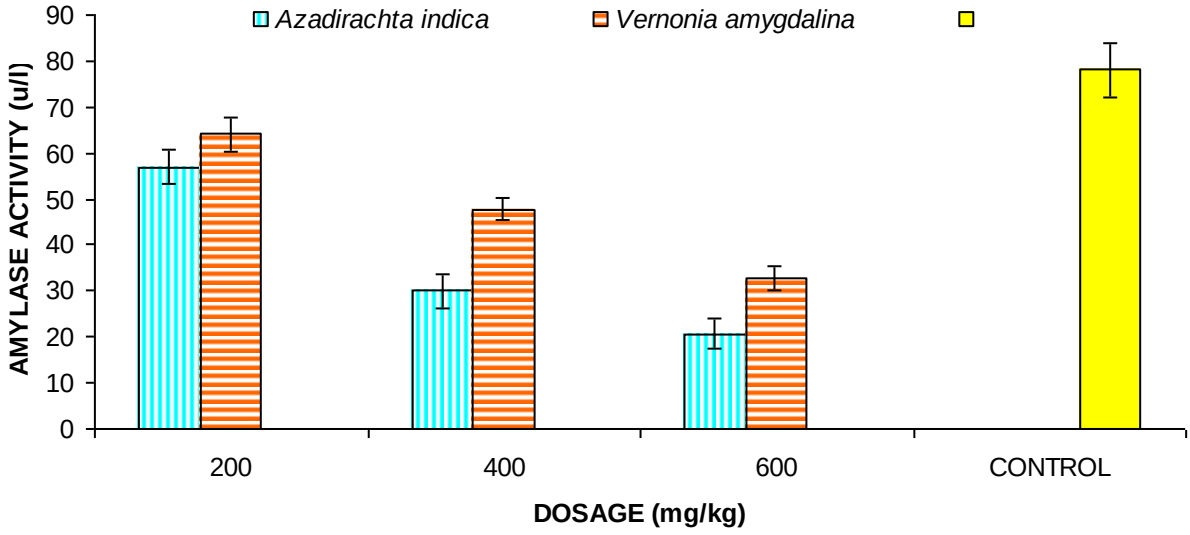

Fig. 3: α-Amylase activities (u/l) in the animals that received leaf-extracts of Azadirachta indica and Vernonia amygdalina.

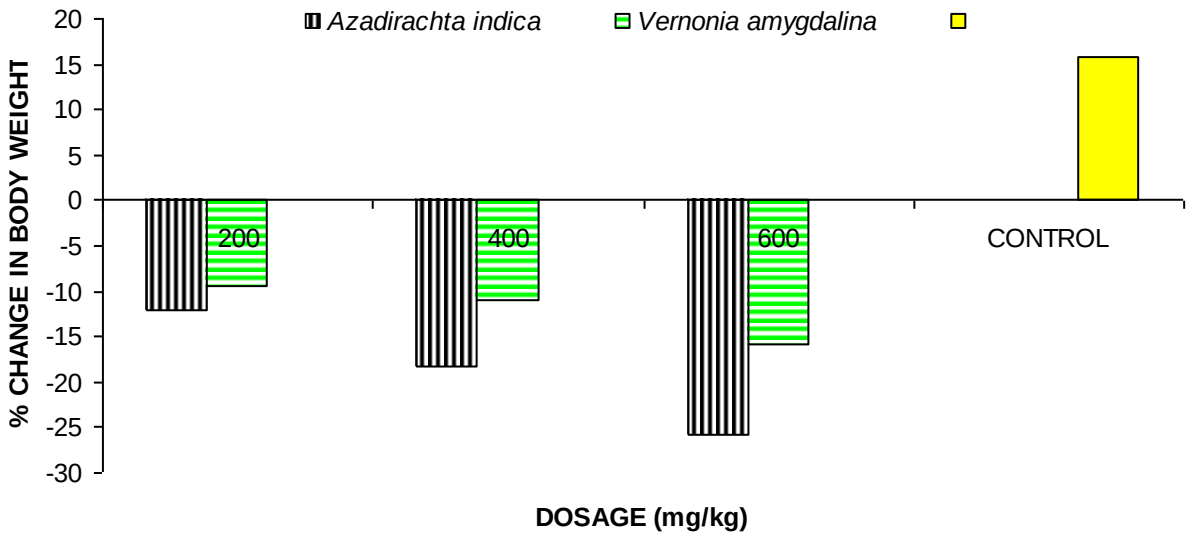

Fig. 4: Percentage change in mean body weights of animals that received leaf-extracts of Azadirachta indica and Vernonia amygdalina.

Discussion

The average activities of alkaline phosphatase (ALP) were significantly ($p<0.05$) higher in the animals that received the extracts at all the stipulated doses, but greater effects were recorded in the groups that received leaf-extract from Azadirachta indica (Fig. 1). Baden et al. [9] reported that elevation of ALP tends to be noted in Paget's disease of bone, obstructive liver disease, hepatotoxicity caused by drug or osteomalacia. The increase in the level of ALP can be attributed to hepatocellular injury by some chemical constituents of the extract [9]. The report of Ahmed and Khater [17] when they treated albino rats with ethanol extract of Ambrosia maritima showed significant increase in alkaline phosphatase levels of the albino rats as the dosage increased and suggested that higher doses of the extract could be hepato-toxic.

The ethanol leaf-extracts of Azadirachta indica and Vernonia amygdalina significantly ($p<0.05$) increased serum GGT activities dose-dependently though the extract of Azadirachta indica exerted more effect (Fig. 2). It has been proved that phytochemical extracts from Azadirachta indica cause increase in liver enzymes [18]. Similar results were obtained when Ali et al. [19] treated mice with ethanol extract of Rhazya stricta and observed a dose-dependent increase of GGT activity in the mice.

The average activities of α-amylase were significantly and dose-dependently lower ($p<0.05$) in the animals that were given the leaf-extracts. The reductive effects in the enzyme activities were more pronounced in the group treated with the extract of Azadirachta indica (Fig.3). Dineshkumar et al. [20] reviewed that neem extract inhibits the action of α-amylase leading to a reduction in starch hydrolysis to maltose and consequentially lower postprandial hyperglycemia. The decrease in the activities of α-amylase might be due to the presence of potential α-amylase inhibitors (alkaloids, flavonoids, terpenoids and glycosides) present in neem extract [21]. Bhat et al. [22] also showed that the ethanol extract of Azadirachta indica caused inhibition of α-amylase. Jamil [23] also recorded a dose-dependent decrease in the activity of amylase when he treated albino rats with ethanol extract of Mitracarpus ovarium and a decrease in amylase activity leads to the formation of abscesses because at low amylase levels, the dead white blood cells are not digested.

The results also showed that the average body weights (g) of the treated animals also decreased significantly and dose-dependently and the reduction was more in the group administered with the extract of Azadirachta indica (Fig. 4). This could be because the extracts delay stomach empting leading to more slow absorption of food substance, which results to loss of appetite and decrease in the weight [24]. Ethel et al. [25] observed that both ethanol and ether extracts of fresh leaves of the African marine plant caused reduction in the average weights of guinea pigs treated with the extracts. The decrease in body weights may be linked to an observed reduction in feed and water intake [26].

Conclusion

The study showed that ethanol extracts of Azadirachta indica and Vernonia amygdalina exerted significant dose-dependent elevations on alkaline phosphatase and gamma-glutamyl transferase activities and showed dose-dependent reductions on α-amylase activities in albino rats. Both also decreased the body weights of the animals at all the stipulated doses. However, the effects were more in the groups administered with the extract of Azadirachta indica. The ethanol extract of Vernonia amygdalina exhibited less toxic potentials than that of Azadirachta indica. Care should be taken in the use of the extracts for therapeutic purposes to obviate possible hepato-biliary diseases.

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