

Total serum protein and albumin levels of Wistar rats on administration of methanol-ethanol (1:1) leaf extracts of *Anacardium occidentale* and *Jatropha tanjorensis*

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ABSTRACT

The effect of methanol-ethanol (1:1) leaf extracts of *Anacardium occidentale* and *Jatropha tanjorensis* on total serum protein and albumin in Wistar rats was investigated. The leaves were prepared and extraction done. The low dose and high dose were established as 10% and 20% respectively after LD₅₀ was determined for methanol-ethanol extracts of *Jatropha tanjorensis* and corresponded to 381 mg/kg bwt and 762 mg/kg bwt respectively. Then 25 female rats weighing 100 -150 g were divided according to body weight into 5 groups of 5 rats each. Group I was the normal control (NC) and animals were given normal feed and water *ad libitum*. Groups II and III were given low (381mg/kg bwt) and high doses (762mg/kg bwt) respectively of *Anacardium occidentale*, while groups IV and V were given low and high doses respectively of *Jatropha tanjorensis*. After 14 days of administration, serum was collected via cardiac puncture and used for analysis of total serum protein and albumin. The results revealed no significant ($P \geq 0.05$) difference in total serum protein (TP) levels in all the test groups, but the albumin levels were significantly ($P < 0.05$) increased in groups III and V and decreased in group IV when compared with the control. Conclusively, the research showed that methanol-ethanol (1:1) extracts of *Anacardium occidentale* and *Jatropha tanjorensis* had no effect on serum total protein but serum albumin was altered either downwards when in low dose or upwards when high doses of the extracts were administered. It is possible that at selected doses, the plant extracts maybe used to improve transportation of minerals, hormones, bilirubin, fatty acids and pharmaceuticals to sites of action. It may also be used to improve the oncotic pressure of the blood as well as correct cases associated with altered serum albumin.

Keywords: hypoalbuminemia, hyperalbuminemia, transport protein, kidney, blood

INTRODUCTION

Plants form an integral part of traditional medical practices in all cultures worldwide and a sizeable portion of the world population use plants for prevention and management of different kinds of ailments. The rural population in particular who do not have access to primary healthcare, either as a result of

non-availability or inability to afford it depend solely on plants remedies for their health problems [1]. Many of the plants used in ethno-medicine have been found to contain useful therapeutic substances and a good number of them have found their way into orthodox medical practice. For instance, morphine,

digoxin, quinine, etc., which are very useful in the managements of diseases that have defied current therapeutic options [2]

. The increasing discovery of more medicinal plants demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physicians and patients make wise decision before using them. Green plants are most significant in their role as producers of food. From them directly or indirectly come animal foods. Proteins, carbohydrates and fats as well as accessory nutrients such as vitamins and minerals are all made available to man and other animals through green plants. Plants are also used as medicine. *Jatropha tanjorensis* (*J. tanjorensis*) belongs to the family, Euphorbiaceae [3]. *J. tanjorensis* is a natural hybrid between *J. curcas* and *J. gossypifolia* [4]. It is a native of Central America and has become naturalized in some tropical and subtropical countries, like India, Nigeria and Canada [4]. Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetable and as medicine [5]. *J. tanjorensis* leaf is commonly consumed

as vegetable in many parts of Southern Nigeria and is commonly called 'Hospital too far', 'Catholic vegetable' or 'Lapalapa' [6]

Chemotherapy and chemoprophylaxis which form an important and major aspect of disease control in Africa and the world over, is beset with problems which include - resistance to drugs, drug toxicity, protracted treatment protocol and limited repertoire of compounds [7]. Decreased protein levels may be seen in pregnancy, chronic alcoholism, prolonged immobilization, heart failure, starvation and mal-absorption or malnutrition. Increased albumin levels are found in dehydration. Decreased albumin levels are also indicative of liver disease, protein-losing syndromes, malnutrition, inflammatory disease, and familial idiopathy (of unknown cause) dysproteinemia. The aim of this investigation is to evaluate the effect of *Jatropha tanjorensis* and *anacardium occidentale* leaf extracts on the total serum protein and albumin levels of Wistar rats.

MATERIALS AND METHODS

Equipment, Chemicals and Reagents

Water bath (grant model:600303003, USA), Table top centrifuge (labofuge 300 Heraus, model D37520, USA), Thermocool freezer (model TH 170, China), Weighing balance (Denver, model:IR-30, USA), and Spectrophotometer (Jenway, model 6405, Japan).

The chemicals methanol, ethanol and diethyl ether were purchased from loba chemical Ltd., Nigeria. All chemicals and used in this research were of analytical grade,

Plant materials

The fresh leaves of *Anacardium occidentale* and *Jatropha tanjorensis* were obtained from Okuku village in Yala Local Government Area of Cross River State, Nigeria. The plants were identified and authenticated by the Department of Botany, University of Calabar.

Extract Preparation

The fresh leaves of *Anacardium occidentale* and *Jatropha tanjorensis* were collected and air-dried under room temperature at Medical Biochemistry Laboratory, Cross River University of Technology (CRUTECH) Okuku Cross River State. The dried samples were ground into fine, flour-like powder or constituency using a Q-link electric blender (model QBL-18L40, China) and stored in air-tight containers.

One hundred grams of *Anacardium occidentale* powder was weighed using an electric weighing balance and soaked in 1000mL of the mixture of methanol-ethanol at ratio 1:1. The mixture was stirred for proper mixing of solvent with powder, then poured into air-tight container. The container with the mixture was kept under room temperature for 48h. *Jatropha tanjorensis* was also

subjected to the same treatment given to *Anacardium occidentale*.

Filtration and Concentration of plant extracts

The mixtures were first filtered using cheese material or cloth, followed by Whatman no.1 filter paper (24cm). The filtrates were then concentrated under reduced pressure, using a rotary evaporator (model RE52A, China) to 10% of its original volume at 37°C. After which it was concentrated to complete dryness in the water bath, yielding 14.98g of *Anacardium occidentale* and 12.82g of *Jatropha tanjorensis*. The extracts were then stored in the refrigerator

Determination of Lethal Dose (LD₅₀)

The assessment of the lethal dose (LD₅₀), (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD₅₀, therapeutic index and the degree of safety of a pharmacological agent [8].

Twelve (12) female Wistar rats weighing between 100 g-138g were obtained from animal house of the Department of Medical Biochemistry, CRUTECH, Okuku-Campus, Cross River State, Nigeria. The animals were kept in well-ventilated laboratory cages, the animals were fed with standard rat pelleted diet and water and also cleaning of beddings for 3 days, together with administration of *A. occidentale* and *J. tanjorensis* ethanol-methanol (1:1) leaf extracts.

In the determination of the LD₅₀, Lorke's Method was employed. The animals were divided into three groups of three rats each and administered the graded doses of 1600, 2900 and 5000 mg/kg/bwt of

test substance and then observed for 24 hours for behavior as well as mortality.

Group 1: 1600mg/kg per body weight of *Anacardium occidentale* and *Jatropha tanjorensis* extracts was administered to the Wistar rats.

Group 2: 2900mg/kg per body weight of *Anacardium occidentale* and *Jatropha tanjorensis* extracts respectively was administered to the Wistar rats.

Group 3: 5000mg/kg per body weight of *Anacardium occidentale* and *Jatropha tanjorensis* extracts was administered to the Wistar rats. The Wistar rat that was administered 5000mg/kg of *Jatropha tanjorensis* died which indicated that *Jatropha tanjorensis* is of high toxicity than *Anacardium occidentale*. It was calculated to be given 10% of the extracts to low doses and 20% of the extracts to high doses.

Then the LD₅₀ was derived based on the formula:

$$LD_{50} = \sqrt{D_n \times D_{100}}$$

D_n = Highest dose that gave no mortality,

D₁₀₀ = Lowest dose that produced mortality.

Thus,

10% for low dose and 20% for high dose of *J. tanjorensis* determined include:

LD₅₀ = 381 mg/kg for low dose *J. tanjorensis*.

LD₅₀ = 762 mg/kg for high dose *J. tanjorensis* respectively.

Animals

Twenty-five (25) female Wistar rats weighing between 100-150g were obtained from the animal house of the Department of Medical Biochemistry, CRUTECH, Okuku Campus, Cross River State, Nigeria. The animals were kept in a well-ventilated laboratory cages with 12-hours day/night cycles and fed with standard rat pelleted diet and water. Proper hygiene involving cleaning of bedding throughout the duration of the experiment was maintained.

Experimental Design

In the experiment, a total of 25 female Wistar rats were used. The rats were divided into five (5) groups of five (5) rats each.

Group I: Normal control (NC) animals was given normal fed and water only.

Group II: Low dose of methanol-ethanol extract of *Anacardium occidentale* administered to Wistar rats

Group III: High dose of methanol-ethanol extract of *Anacardium occidentale* was administered to Wistar rats.

Group IV: Low dose of methanol-ethanol extract of *Jatropha tanjorensis* was administered to Wistar rats.

Group V: High dose of methanol-ethanol extract of *Jatropha tanjorensis* was administered to Wistar rats.

Blood sample collection

Two weeks after drug administration, the rats were subjected to a 16h overnight fast prior to sacrifice and anaesthetized by diethyl ether (5%) inhalation. They were later placed on a dissecting slab and the limbs pinned down with the aid of

Result of Total protein and albumin of Wistar rats administered methanol-ethanol (1:1) extracts of *Anacardium occidentale* and *Jatropha tanjorensis* are given in Table 1.

The result revealed no significant ($P \geq 0.05$) difference in both total protein (TP) levels in all the test groups compared with both the control and with each other. But the albumin levels were significantly ($P < 0.05$) increased in groups III and V and decreased in group IV when compared with the control. The albumin levels in groups II and IV compared well and so were groups III and V. Therefore, the plant extracts had no effect on total

dissecting pins. A longitudinal incision was made abdominally to the rib followed by transverse incision to the limbs. Blood samples were collected with the aid of sterile syringes and needles through cardiac puncture from the left ventricle and were transferred into well labelled heparin-treated tubes and centrifuged in order to separate the serum from blood cells. The heparin-treated tubes containing serum and packed blood cells were stored at optimal temperature (4°C) until when needed for use.

Estimation serum total protein

Total serum protein was determined using biuret method.

Statistical Analysis

Data obtained was analysed using Microsoft Office Excel 2007 and expressed as mean \pm SEM. The statistical package SPSS version 17.0 using one-way ANOVA (Analysis of variance) was used to establish statistical significance at $P < 0.05$.

RESULTS

protein levels but produced effects on albumin levels of the experimental rats.

Table 1. Administration of methanol-ethanol (1:1) extracts of *Anacardium occidentale* and *Jatropha tanjorensis* on total protein and albumin in Wistar rats

Parameters Group	Total Protein (g/dL)	Albumin (g/dL)
I (NC)	6.68 ± 0.07	2.76 ± 0.10 ^a
II (LAO)	6.79 ± 0.06	2.53 ± 0.07 ^{ab}
III (HAO)	6.77 ± 0.18	3.35 ± 0.16 ^c
IV (LJT)	6.50 ± 0.08	2.41 ± 0.06 ^b
V (HJT)	6.702 ± 0.05	3.54 ± 0.08 ^c

Values are presented as mean ± SEM (n=5)

^{a,b,c} Values with different superscripts are significant at $P < 0.05$

Note: NC= Normal control, LAO= Low dose (*Anacardium occidentale*), HAO= High dose (*Anacardium occidentale*), LJT= Low dose (*Jatropha tanjorensis*), HJT= High dose (*Jatropha tanjorensis*).

DISCUSSION

Proteins are large molecules (complex organic compounds) that consist of amino acids. There are two main types of proteins: those that are made of fiber which forms the structural basis of the body tissues, such as hair, skin, muscles, tendons, and cartilage; and globular proteins (generally water soluble), which interact with many hormones, various other proteins in the blood, including hemoglobin and antibodies, and enzymes. Proteins are needed in the diet to supply the body with amino acids. Ingested proteins are broken down in the digestive system to amino acids, which are then absorbed and rebuilt into new body proteins. A severe loss of protein from the bloodstream into the urine (proteinuria) results in lowering of the protein content of the blood and resulting in fluid retention causing oedema. In this study the methanol-ethanol extracts of *Anacardium occidentale* and *Jatropha tanjorensis* had no effect on serum total protein. One of the most important functions of proteins in the body is to

contribute to the osmotic pressure (the movement of water between the bloodstream and tissues) and this property is exhibited by albumin. Albumin maintains osmotic pressure and helps transport certain blood constituents around the body via the bloodstream. Serum albumin levels can also be used as an indicator of liver function, since it is produced by the liver. There was no clear-cut effect of the plant extracts on serum albumin as low doses of *Anacardium occidentale* and *Jatropha tanjorensis* appear to tilt towards hypoalbuminemia, while high doses of plant extracts favour hyperalbuminemia. Hypoalbuminemia which is low blood albumin, is a well-established risk factor of morbidity and mortality [9], [10]. It can be caused by liver disease, excess excretion by the kidneys (as in nephrotic syndrome), malnutrition and wasting among others [11]. Malnutrition and inflammation also suppress albumin synthesis [12]. The correction of hypoalbuminemia has been

reported to be kidney-protective [10]. Hyperalbuminemia is an increased concentration of albumin in the blood [13]. Chief factors responsible for an increase in albumin level are severe infections, congenital disorders, severe dehydration, hepatitis, malnourishment, chronic inflammatory disease [14]. It is very unlikely that the plant extracts of *Anacardium occidentale* and *Jatropha tanjorensis* may have caused any of these, but it is most likely that the hypoalbuminemia may be due to the fact that the expression of certain agents in

the plants extracts was made possible at low dose, while at high dose of the plant extracts which led to hyperalbuminemia, other agents are allowed to express themselves. It is possible that when the right doses of the plant extracts of *Anacardium occidentale* and *Jatropha tanjorensis* are used, it may enhance normal serum albumin levels. High serum albumin level though has been reported a predictor of favourable response to immunotherapy in autoimmune encephalitis [15]

CONCLUSION

The research showed that methanol-ethanol (1:1) extracts of *Anacardium occidentale* and *Jatropha tanjorensis* had no effect on serum total protein but serum albumin was altered either downwards when in low dose or upwards when high doses of the extracts were administered. It is possible that at selected doses, the plant extracts maybe

used to improve transportation of minerals like calcium, potassium, sodium, as well as hormones, bilirubin, fatty acids and pharmaceuticals to sites of action. It may also be used to improve the oncotic pressure of the blood as well as correct cases associated with altered serum albumin.

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