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Renal function indices in Wistar rats administered methanol-ethanol (1:1) leaf extracts of *Anacardium occidentale* and *Jatropha tanjorensis*

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ABSTRACTS

The effect of methanol-ethanol (1:1) leaf extracts administration of *Anacardium occidentale* and *Jatropha tanjorensis* on renal function indices in Wistar rats was investigated in this study. 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 urea and creatinine. The results revealed a significant ($P<0.05$) increase in creatinine levels of the test groups when compared with the normal control (NC). The serum creatinine levels of group II and V compared well with each other. However, serum urea levels were significantly ($P<0.05$) decreased in groups IV and V while groups II and III showed no significant difference ($P>0.05$) compared with the control. Conclusively, the study showed that combined methanol-ethanol (1:1) leaf extracts of *Jatropha tanjorensis* have the potential to improve kidney function, but *Jatropha tanjorensis* and *Anacardium occidentale* extracts affect serum creatinine levels possibly in a similar fashion as cimetidine, trimethoprim, corticosteroid or vitamin D metabolite.

Keywords: Urea, creatinine, plant extract, combined-solvent extraction and kidney.

INTRODUCTION

The benefits derived from using medicinal herbs in disease management are immense. Plant medicine is relatively safe, more affordable and sometimes offer better therapeutic value than synthetic drugs. This is because human beings have co-evolved with plants over the past decades [1]. Medicinal plants are the sources of many important scientific drugs of the modern world [2]. *Jatropha tanjorensis* (*J. tanjorensis*) belongs to the family, *Euphorbiaceae* [2]. *J. tanjorensis* is a natural hybrid between *J. curcas* and *J. gossypifolia* [3]. It is a native of Central America and has become naturalized in some tropical and subtropical countries, like India, Nigeria and Canada [3]. Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetable and as medicine [4]. *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' [5].

J.tanjorensis popular as a natural remedy against diabetes in Southern Nigeria [6]. The leaf is used as heart tonic and remedy for hypertension in some parts of Nigeria [5]. The leaf extract has hypoglycemic activity and the aqueous leaf extract exhibit antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [7].

Anacardium occidentale commonly called cashew is native to Brazil, and belongs to the family of Anacardiaceae. Vietnam, Nigeria, India and Cote d'Ivoire are major producers of Cashew [8].

The medicinal parts of cashew are the nuts, leaves, stem bark, root and gum. It exhibits antimicrobial and antifungal activities, with properties against worms and protozoa. In African herbal medicine the leaves are used to treat malaria [9].

The therapeutic values of most of these herbs are indisputable but their toxicities sometimes limit their clinical uses. Thus, the toxicity profile of these herbs must always be considered especially as the doses and dosing regimens of their preparations are not usually determined [10]. The world is beset with problems which include - resistance to drugs, drug toxicity, protracted treatment protocol and limited repertoire of compounds [11]. The problem of drug toxicity and resistance is very pronounced in the management of many certain diseases, e.g. hypertension. Also, the problem of non-compliance where patients are usually unwilling to adhere to prescriptions, requiring the combination of several drugs which can be taken care of by the potential wholistic medicinal effects that may be elicited by these plants' extracts. Although, herbal medicines are widely preferred in the developing countries because of their availability and affordability, though, they are not without side effects, some of which are so severe that the plants may be considered as poisonous [12]. Medicinal plants can also be a source of exposure to toxic elements depending on their origin and nature. The preparation and use can be harmful to human health, which has encouraged researchers to investigate medicinal plants [13]. Some medicinal plant preparations from the shoot of *Anacardium occidentale* and leaf of *Carica papaya* have been reported to cause acute renal failure hence increasing the progression of kidney related challenges. Chronic Kidney Diseases (CKD) for example affects about 10% of the world's adult population and is among the top 20 causes of death worldwide [14]. One of the most important functions of the kidney is filtration and excretion of nitrogenous waste products from the blood [15]. The measurements of urea and creatinine serve as indicators to acute renal injury [15].

Medicinal plants help to ameliorate the effect of oxidative stress which is a major contributing factor in kidney damage [16].

The extracts of *A.occidentale* and *J. tanjorensis* leaves have been used in the management of different health disorders in Nigeria and the world at large. These and other reports have necessitated the assessment of combined methanol-ethanol (1:1) leaf extracts of *A. occidentale* and *J. tanjorensis* on renal function indices of Wistar rats at selected dose levels.

MATERIALS AND METHODS

Equipment, Chemical 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 reagents 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 [17].

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:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

D₀ = 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.

BIOCHEMICAL ASSAYS

The biochemical assay of serum urea and creatinine concentrations for kidney function determination were done using Dialab and Agappe kits respectively. Urea concentration was measured spectrophotometrically at 578nm and the increase in absorbance was proportional the urea concentration in the sample. Creatinine was measured spectrophotometrically at 520nm based on the principle of its reaction with picric acid to produce a coloured, creatinine alkaline picrate.

STATISTICAL ANALYSIS

Data obtained was analysed using Microsoft Office Excel 2007 and expressed as mean ± 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

The results of kidney function indices of Wistar rats administered methanol-ethanol (1:1) extracts of *Anacardium occidentale* and *Jatropha tanjorensis* are given in Table 1. The results revealed a significant ($P < 0.05$) increase in creatinine levels of the test groups when compared with the normal control (NC). The serum creatinine levels of groups II and V also compared well with each other. However, there was a significant ($P < 0.05$) decrease in the serum urea levels of groups IV and V following the administered extracts when compared with the control, but no significant ($P \geq 0.05$) difference was observed in serum urea levels of groups II and III compared with the control.

Table 1. Kidney function indices of Wistar rats administered methanol-ethanol leaf extracts of *Anacardium occidentale* and *Jatropha tanjorensis*.

PARAMETERS GROUP	CREATININE	UREA
I (NC)	2.62± 0.02 ^a	113± 1.47 ^a
II (LAO)	2.76± 0.02 ^b	111± 0.63 ^a
III (HAO)	2.86± 0.02 ^c	113± 2.04 ^a
IV (LJT)	2.94± 0.02 ^d	90.0 ± 0.28 ^b
V (HJT)	2.76 ±0.02 ^b	91.0 ± 0.00 ^c

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

^{a,b,c,d}Values with different superscript are statistically different 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

The biochemical indices evaluated in the study are useful parameter to indicate impairment in the functional capacity of the kidney [18]. Renal function tests are usually required to assess the normal functioning units of the kidney - the nephron.

Urea is the major nitrogen containing metabolic product of protein catabolism. The significant reduction in serum urea concentration following the administration of methanol-ethanol (1:1) leaf extracts of *Jatropha tanjorensis* at various doses may be attributed to improvement in the physiological excretion of urea caused by the plant extract in this study. The *Anacardium occidentale* extract showed no significant difference which may suggest no effect on the functional integrity of the kidney

Serum creatinine is an important indicator of renal health because it is an easily measured by-product of muscle metabolism that is excreted unchanged by the kidneys. If the filtration in the kidneys is deficient creatinine blood levels rise. The consistency of endogenous creatinine production and its release into the body fluids at a constant rate and constancy of plasma levels of creatinine 24 hours of the day, makes creatinine a useful endogenous substance where clearance may be measured as an indication of creatinine content [19]. The significant increase in serum creatinine following the administration of methanol-ethanol extracts of *Jatropha tanjorensis* and *Anacardium occidentale* may be an indication of glomerular and tubular mass dysfunction. Renal damage reduces the functioning of the tubular mass and may seriously affect the regulatory function of the kidney. However, it is unclear why on one hand urea levels were normal or reduced in the blood but on the other hand creatinine was increased. It can be presumed that the plant extracts may have rather caused increased breakdown of creatine phosphate in the muscle hence leading to increased production of creatinine and its presence in the blood rather than reduced kidney function. Drugs such as cimetidine, trimethoprim, corticosteroid, vitamin D metabolite etc have been reported to increase plasma creatinine without altering its glomerular filtration, modifying the production rate and the release of creatinine [20]

CONCLUSION

The results from the present study involving the use of combined methanol-ethanol extracts of *Jatropha tanjorensis* and *Anacardium occidentale* in Wistar rats showed that *Jatropha tanjorensis* has the potential to improve kidney function, but *Jatropha tanjorensis* and *Anacardium occidentale* affect serum creatinine levels possibly in a similar fashion as cimetidine, trimethoprim, corticosteroid or vitamin D metabolite.

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