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CREATININE, UREA AND URIC ACID LEVELS IN ALBINO RATS TREATED WITH LEAF EXTRACT OF *CANJANUS CAJAN* (PIGEON PEA).

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ABSTRACT

Several parts of *Cajanus cajan* have been utilized traditionally in Eastern Nigeria in treatment and management of several ailments such as liver, heart and kidney related diseases. This present research was carried out to examine the effect of oral administration of aqueous extract of fresh leaves of *Cajanus cajan* on creatinine, urea and uric acid concentrations in albino rats. The research was performed with twenty-five adult male albino rats distributed into five groups (A, B, C, D and E) with five rats in each group. The animals in groups A, B, C and D were administered orally with aqueous extract of fresh leaves of *Cajanus cajan* using doses of 150,300, 450 and 600mg/kg respectively for seven consecutive days, while group E served as the control. There was a decrease in physical activities and rate of feed and water intake in the test groups when compared with the control. There was a significant decrease ($P< 0.05$) in concentrations of creatinine, urea and uric acid in the animals given the extract when compared to the control group. These effects were found to be dose dependent. The result obtained from this research suggests that aqueous extract of fresh leaves of *Cajanus cajan* possess the potential to lower serum concentration of creatinine, urea and uric acid. This may be responsible for the utilization of the leaves of *Cajanus cajan* in management and treatment of kidney related diseases.

Keywords: Creatinine, Urea, Uric acid, Albino rats and *Canjanus cajan* (pigeon pea)

INTRODUCTION

Throughout history, plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use. It has been reported that plants produce a vast array of secondary metabolites for defence against environmental stress or other factors like pest attacks, wounds and injuries.This secondary metabolite produced by the plant has been found to be useful in various therapeutic aids in medicine from time immemorial [1 and 2]. Orthodox medicine through is generally preferred and acceptable, traditional medicine is still very much relied on all over the world [3 and 4], this is common in developing countries where the cost of orthodox medicine is astronomical and unaffordable to a large stage of the populace [5]. According to the world health organisation, 80% of the

population in developing countries depends on traditional medicine for their primary health care and about 85% of such traditional medicine involves the use of plant extract for their production [6]. In 2001, it has been reported that researchers identified 122 compounds used in modern medicine, where all derived from ethnomedicinal plant source; 80% of these compounds are identical or related to the current use of active elements of the plant [7].

Medicinal plants are plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesise metabolites to produce useful drugs [8]. This herbal medicine does not differ greatly from conventional drugs in terms of how they work, this enables herbal medicine to be as effective as conventional medicines but also give the same potential to cause harmful side effects [9 and 10]. In Nigeria, there is existence of many medicinal plants which possess many therapeutic effects on diseases [11]. All these plants (Medicinal plants) contains chemical compounds (Phytochemicals) which are divided into primary and secondary metabolites, it is the secondary metabolites that have therapeutic actions in human and are refined to produce drugs example morphine and codeine from poppy, quinine from cinchoma plants and so on [12]. The use of herbs as medicines has played on important role in nearly every culture on earth, including Asia, Africa, Europe and Americans. Several herbs can help to reduce high blood cholesterol concentration and blood sugar level; example ginseng plant and milk thistle which is often used for treating liver on gall bladder diseases, cirrhosis and hepatitis and may help protect the liver from damage due to alcoholism [13].

Cajanus cajan is one of the medicinal plants used for food, medicinal purposes and as a source of drugs, it belongs to the family of *fabaceae* and its class is *magnociopsida* (dicotyledous) [14]. *Cajanus cajan* is the most important leguminous grain crop of rain-feed agriculture in semi-tropics. It is a perennial plant and it is popularly called pigeon pea, it is a multi-purpose plant that is extensively eaten as food and for medicinal purposes [15].

Cajanus Cajan is cultivated in ancient Egypt, Africa and Asia since prehistoric times and was later introduced to America. The major producer is India. It is essentially a plant of the semi-dry lowlands [16]. *Cajanus Cajan* has been used widely for many years for treating diabetes, sores, skin irritations, hepatitis, measles, jaundice, dysentery, expelling bladder stores and stabilising menstrual period. In Trinidad and Tobago, the leaves of *Cajanus cajan* are used in food poisoning as colic and in constipation [17]. Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body (depending on muscle mass). Serum creatinine (a blood measurement) is an important indicator of renal health because it is easily measured as a by-product of muscle metabolism that is excreted unchanged by

the kidneys [18]. The typically human reference ranges for serum creatinine are 0.5-1.2mg/dl (about 45-90µ mol/l) for adults male, 0.4-1.1mg/dl (about 60-110µmol/l) and 0.0 – 0.7mg/dl for children up to 12 years of age. Urine creatinine ranges for adult male is 20-25mg/kg/day and for adult female 15 – 20mg/kg/day [19]. Increased creatinine levels are seen in impaired renal function, chronic nephritis, shocks and muscle atrophy can result in decreased creatinine level.

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen and hydrogen with the formula C5H4N4O3. It forms ions and salts known as urates and acid urates such as ammonium acid urate [20]. In human blood plasma, the reference range of uric acid is typically 3.4 – 7.2mg/dl (200 - 430µmol/l) for an adult male, 2.4 – 6.1mg/dl for an adult female (140 - 360µmol/l) and children range from 3-4mg/dl. Drugs such as Thiazide diuretic can increase uric acid level by interfering with renal clearance [21]. Urea (Carbamide) is an organic compound with the chemical formula CO(NH2). The molecule has two–NH2 groups joined by a carbonyl (C= 0) functional group. The liver forms urea by combing two ammonia molecules (NH3) with a carbon dioxide (CO2) molecule in the urea cycle. 7 to 20mg/dl (2.5 to 7.1mmol/l) is considered normal range. For adult male is 8 to 20mg/dl, adult female 6 to 20mg/dl and children 5 to 18mg/dl.

AIM/OBJECTIVE

The aim of this research was to evaluate the effect of aqueous extract of fresh leaves of *Cajanus cajan*on on the kidney of albino rats, by measuring the levels of creatinine, uric acid and urea after treating the albino rats with the aqueous extract of *Cajanus cajan*.

MATERIALS AND METHODS

Collection of Samples

Fresh leaves of cajanus cajan were collected from Ndulo Ukwagba Ngbo, Ohaukwu Local Government Area of Ebonyi State. Twenty–Five albino rats were purchased from Zoology Department of University of Nigeria, Nsukka, Enugu State, and transported to Biochemistry Animal House Ebonyi State University, Abakaliki.

PREPARATION OF EXTRACT

One hundred and thirty gramme of fresh leaves of *Cajanus cajan* was washed with clean water and air dried at room temperature and was later homogenised after which 300ml of distilled water was added and allowed to soak for 1h. It was then filtered and squeezed out using a lilac sieving cloth to get a greenish aqueous extract.

ANIMAL HANDLING AND TREATMENT

Animal Grouping

They were kept in steel cages and were fed with water and poultry growers mash. After acclimatisation stage, which lasted for 4 days, the rats were assigned into 5 groups, A, B, C, D and E, each containing 5 rats. All animals were allowed free access to water and feed (Grower mash).

Measurement of weight of animals

The weights of animals were taken on a daily basis using a weighing balance and the exact volume of the aqueous extract to be administered were calculated based on their body weight.

Administration of Extract

The plant extracts were administered to the animals orally using the 2ml syringe. Groups A – D were given the extract of *cajanus cajan* with doses of 450, 150, 600 and 300mg/kg respectively for seven consecutive days while animals in group E is the control.

COLLECTION OF BLOOD FROM ANIMALS

After seven days of administration of the sample to the albino rats, they were starved over right, under a mild anaesthesia using chloroform, blood sample were collected from the albino rat by cardiac puncture. The blood samples were put into sterile bottle free of anticoagulant.

PREPARATION OF SERUM

Three mills of blood were collected from the animal (albino rat) in sterile specimen bottle and allowed to cloth. It was centrifuge at 3000rpm for 5minutes and the serum separated from the plasma with the aid of a Pasteur pipette.

MEASUREMENT OF PARAMETERS

Urea

Urea was measured according to the method of Berthelot reagent as described by [22].

Creatinine

Creatinine was measured according to the method of [23], as described by [24].

Uric Acid

Uric acid was measured according to the method of Enzyme Colorimetric as described by [25].

RESULTS

PHYSICAL OBSERVATION

The rats in groups A, B, C and D showed a reduction in physical activities and feed consumption unlike those in group E, the control.

CHANGES IN AVERAGE BODY WEIGHT (g) OF RATS DURING SEVEN DAYS OF EXTRACT ADMINISTRATION

The change in the average body weights of the animals is presented in table 1. There was no significant difference (P<0.05) between changes in average body weight of the albino rats in the groups given the extract and the control.

Table 1: The average body weight of the rat during 7days of administration of extract.

Days	Group A	Group B	Group C	Group D	Group E
1.	112.42±7.11	124.24±4.69	116.22±4.14	92.24±4.24	124.25±2.96
2.	116.25±6.91	116.24±5.13	136.34±4.07	84.32±4.69	120.28±4.97
3.	108.24±6.45	120.20±4.97	116.26±4.11	96.38±4.67	132.30±5.19
4.	124.14±6.36	120.14±4.75	104.24±4.21	98.10±4.22	127.34±3.49
5.	127.24±5.53	111.14±5.34	113.14±3.24	85.30±2.42	132.18±4.33
6.	114.14±6.89	119.32±5.32	115.22±2.97	77.14±4.37	134.14±4.12
7.	125.18±6.12	126.22±4.03	117.28±4.97	80.30±0.49	122.10±2.10

Values are mean ± standard deviation; n=5

CONCENTRATION OF URIC ACID, CREATININE AND UREA OF THE RATS AFTER SEVEN DAYS OF TREATMENT

The results obtained from the parameters are shown in 2. The concentration of creatinine, urea and uric acid in the serum of the rat administered the extract were significantly lower (P<0.05) than in the control group. The decrease in the concentration of creatinine, uric acid and urea were found to be dose dependent.

Table 2: The average concentration of urea, creatinine and uric acid level

Animal group	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl
A	7.93±0.53	4.99±0.52	0.87±0.26
B	6.89±1.35	4.47±0.56	1.02±0.39
C	5.49±0.99	3.70±0.21	0.65±0.23s
D	3.92±0.14	2.03±0.47	0.39±0.22
E	12.52±1.02	8.49±0.69	1.13±0.19

Values are mean ± SD; n = 5.

DISCUSSION AND CONCLUSION

DISCUSSION

The biochemical mechanism underlying the observed decrease in the physical activities of rats administered with the aqueous extract of *Cajanus cajan* is yet not fully understood at this stage of the research. However, such decrease may be as a result of chemical constituents of the extract administered to the animals as suggested by [26], who observed similar effect when he treated adult male albino rats with the leaf extract of *Ageratum Conyzoides.* Phytochemicals such as alkaloids, tannins, saponins and flavonoids have been reported to decrease the physical activities of laboratory animals [27]. However, it could be attributed to the changes in metabolic activities of the treated animals elicited by constituents of the extract. Plant extracts have been reported to contain chemical compounds that lower appetite for example tannins present in leaf extract can lower feed and water intake [28]. Further investigations are required to fully explain the actual biochemical mechanism.

Serum creatinine is an indicator of renal health because it is an easily measured by the product of muscle metabolism that is excreted unchanged by the kidney. If the kidney is deficient in its filtration ability, creatinine blood level rises. The creatinine levels of the rats administered with the aqueous extract of fresh leaves of *Cajanus cajan* did not decrease significantly (P>0.05) when compared to the control (table 2). This result is in accordance with the result obtained by [26], when he treated albino rats with aqueous extract of *Ageratum Conyzoides*. Since the result of this research showed a decrease in creatinine level, the extract is assumed to be nephroprotective. However, this decrease in creatinine level may be attributed to the chemical constituent of the extract such as flavonoids which shows that the plant extract is good for management of cardiovascular diseases and oxidative stress [29].

In a similar research carried out by [30], on the effect of the mixture of *Gongronema latifolia, Ocimum gratissimum* and *Vernonia amygadalina* (GOV) showed that there was a significant increase in creatinine and urea concentration. Urea which plays an important role in the metabolism of nitrogen-containing compounds by animal and is the main nitrogen-containing the substance in the urine of mammals, when urea is high in the blood can result in tissue breakdown e.g. haemorrhage. From the present research, the result obtained showed a decrease in urea concentration of treated rats though the decrease was not significant ($P>0.05$) when compared to the control. This result is in line with the result obtained by [31 and 32] when they used *Rauwolfia vomitoria* and *Allium sativum* respectively to treat male rats.

The biochemical explanation for the insignificant decrease ($P>0.05$) in the level of uric acid in treated rat when compared to control (table 2) suggests that the decrease maybe as a result of the presence of anthocyanins, a chemical constituent present in the leaf extract of *Cajanus cajan*. Uric acid being the product of the metabolic breakdown of purine nucleotides when high in blood can lead to gout. Since there was a decrease in the level of uric acid, the anthnocyanins present in the extract may have a positive influence in preventing inflammation (gout arthritis, rheumatoid arthritis) and subsequent blood vessel damage [33]. This result is in line with the result obtained by [33], when he used *Carica papaya* to investigate the protective effect of aqueous extract of *Carica papaya* seeds in paracetamol induced nephrotoxicity in male Wister rats which showed a decrease in concentration of uric acid, creatinine and urea.

CONCLUSION

The present study showed that the aqueous leaf extract of *Cajanus cajan*is capable of lowering kidney function parameters. From the result it showed that extract of *Cajanus cajan*has a potential to be nephron protective. This may partly explain the use of the leaves in management and treatment of renal disorders.

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