European Journal of Biology and Medical Science Research

Vol.6, No.3, pp.1-9, July 2018

Published by European Centre for Research Training and Development UK (www.eajournals.org)

EFFECT OF CASSAVA (*MANIHOT ESCULENTA*, CRANTZ) EXTRACT ON BIOCHEMICAL PROFILE OF INFECTED ALBINO RATS

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ABSTRACT: *This study is designed to evaluate the effect of ethanolic extract of Cassava (Manihot esculenta, crantz) on biochemical profile of Albino Rats. Four varieties of cassava leaves were used: two for sweet cassava (TMS 98/0505, TME 419) and two for bitter cassava (TMS 98/0002, NR 8082). Eighty Wistar Albino Rats of both sexes weighing 150-200g were used. The animals were orally infected with human intestinal parasites. They were randomly divided into 5 groups and subjected to different treatments. The results showed that sweet cassava varieties caused significant decrease (P<0.05) in the valves of ALT, ALP and CRT, while bitter cassava varieties caused significant increase (P<0.05) in the levels of ALT, ALP and CRT in the treated groups when compared with control. The ethanolic extract of the Sweet Cassava Varieties protected the liver and kidney of Albino Rats from damage caused by intestinal parasitic infections. These Varieties are useful phytomedicines for controlling intestinal parasite infections.*

KEYWORDS: Manihot Esculenta Crantz, Biochemical Profile, Albino Rats, Ethanolic, Human Intestinal Parasites, Phytomedicines

INTRODUCTION

Cassava (*Manihot esculenta, crantz)* is a major staple food in developing countries providing a basic diet for over 800 million people around the world (FAO, 2007). It is the third largest sources of carbohydrate within the tropical regions after rice and corn (Ceballos *et al.*, 2004).

Cassava originated from South America and belongs to the family Euphor biacea and genus Manihot. There are several thousand varieties of cassava but they are classified as sweet or bitter depending on the level of toxic cyanogenic glycosides (Oyewole and Afolabi, 2001)

Cassava is also known as Manioc, Tapioca or Mandioca, and possesses some unique and remarkable characteristics. The crop is hardy and can survive adverse conditions such as infertile soil, drought, pests and diseases (El-Sharkawu, 2003). The plant is easy to grow and can be safely left in the ground for several months making it an excellent food security crop.

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

Cassava's combined abilities to produce high yields under poor conditions and store its harvestable portion underground until needed, makes it a classic "food security crop" (Nweke, 2003).

Cassava leaves are by - products of cassava root harvest. These leaves are regarded as waste and are usually allowed to rot. This is in spite of the potential values of this by – product. Research has shown that cassava leaves contain nutrients such as vitamins, A, B2, B6, C, Carotenoids, minerals, carbohydrates and different kinds of protein (Achidi *et al.*, 2005).

They also contain anti-nutrients, such as, cyanogenic glycosides (linamarin and loutaustralin), coumarins (scopoletin, esculetin and their glycosides; scopolin and esculin), phenolic compounds such as tannin flavonoids etc. These anti-nutrients are characterized by diversities of biological (toxicological) effects including carcinogenic neurotoxic, hepatotoxic, nephrotoxic, and immunotoxic effects (Aiofe and Richard, 2004). Studies also showed that Manihot *esculenta, crantz* extact has been used as analgesic, anti hemorroid, anti-inflammatory, anti-pyretic, anti-diaorrhea, antioxidant and anti-helmintic (Jayasiri *et al.*, 2011).

Most importantly, cassava leaves have been used by traditional healers with success in the control of gastro-intestinal parasitism (Sokerya and Preston, 2003).

Therefore, the aim of this study is to assess the effect of the ethanolic extract of various species of cassava leaves using biochemical parameters.

MATERIALS AND METHODS

Study Site

The study was carried out in Owerri, the capital city of Imo State, Nigeria. Imo State is located in the South Eastern Region of Nigeria and is one of the 36 States of Nigeria. It lies between latitude 6^{o} 35^{I} and 7^{o} 28^{I} north of the equator and longitude 5^{o} 10^{I} and 6^{o} 0^{I} east of the meridian (NIMET, 2007).

The area experiences the humid semi hot equatorial climate. The rainfall is heavy with an average annual rainfall of 2000-2400nm. Rainfall distribution is bimodal with peaks in July and September and a 2 weeks break in August. An average annual temperature above $20^{o}C(68^{o}F)$ creates an annual relative humidity of 75%. The area is in tropical rain forest zone and is richly endowed with fertile land suitable for growth of arable crops (Imo ADP, 2004).

Ethical Clearance

Experimental procedures and protocols used in this study were approved by the ethics committee of Imo State University, Owerri, Nigeria.

Collection of Plant Materials

Four different species of cassava (*Manihot esculenta, crantz),* two for sweet cassava (TMS 98/0505, TME 419) and two for bitter cassava (TMS 98/0002, NR 8082) were used for the study. Fresh leaves of each of the plant species were collected from the farms and authenticated by a botanist. The leaves were blended into fine powder with an electric blender, oven dried

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

and washed. The washed leaves were dried at room temperature and stored in an air tight container.

Preparation of Plant Extract

The leaf powder was extracted with 90% ethanol using soxhlet apparatus at 50-55^{o}C for 3 days. The extract was concentrated in a ventilated oven at 45^{o}C for 2 hours.

METHODS

Acute Oral Toxicity Study

Acute oral toxicity study for the plant extracts were carried out using the modified method of Dietrich Lorke, (1983). The study revealed that the administration of ethanolic leaf extract of *Manihot esculenta crantz* was safe up to a dose of 2000mg/kg. No death was observed up to this dose and the experimental animals were physically active.

Animals

Healthy adult wistar Rats of either sex weighing 150 – 200g were used for the study. They were caged in polyvinyl wire mesh cages in the animal house of the department of Animal Science, Imo State University, Owerri. They were maintained under standard laboratory conditions (12 hours light and dark cycle and temperature of 27^{o}C±2^{o}C and humidity (60±10%) with free access to food and were *ad libitum*. Animals were acclimatized to laboratory conditions for 14 days before the experiment.

Experimental Design

A total of eighty (80) Adult Wistar Albino Rats were maintained under standard laboratory conditions. They were orally infected with one hundred (100) infective larvae (L_3)of human intestinal parasites (*Ascaris lumbricoides and Ancylostoma duodenale)* obtained from coprocultures. Infections were confirmed before the beginning of the experiment, by collecting faecal samples from each animal using McMaster technique. Only infected animals were used for the experiment. In each plant treatment trial, the experimental animals (n=20) were divided into five groups (n=4) and subjected to different treatments with single dose of plant extracts and/or commercial anti-parasitic as follows, 1st, 2nd and 3rd groups received single doses of 100mg/kg, 200mg/kg and 400mg/kg, respectively of respective plant extracts while the 4th group acted as positive control group and was given single dose of levamisole 7.5mg/kg and 5th group served as negative control group and received no treatment.

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Blood Sample Collection

At the end of the experiment, the animals were anaesthetized under diethyl ether. Blood was collected from each animal by cardiac puncture using sterile needles and syringes.

Biochemical Analysis

Blood samples for biochemical analyses were collected in plain containers, allowed to clot and were then centrifuged to obtain the serum. Analysis was done using automated Biochemistry analyzer (VITRO SCIENT VS 10).

The biochemical parameters analyzed include, Alanine amino transferase (ALT) Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN) and Creatinine (CRT).

Statistical Analysis

The statistical analyses were performed with statistix 8 software package. All data were normalized using (log + 1) transformation before submitting to general linear model procedure analysis of variance. The differences between the means were considered significant at $P<0.05$.

RESULTS

Biochemical Changes

The values of biochemical parameters obtained in this study are as shown in Tables 1-4.

Treatment with TMS 98/0505 and TME 419 (sweet cassava varieties) showed dose dependent significant decrease ($P<0.05$) in ALT, ALP and CRT values at 100 and 200mg/kg, while treatment with TMS 98/0002 and NR/8082 (bitter cassava varieties) showed dose dependent significant increase ($P<0.05$) in ALT, ALP and CRT values at 400mg/kg when compared with the control. AST and BUN values were not significantly affected. All serum biochemical values were within the reference ranges for rats. The reference ranges of AST, ALT, ALP, BUN, and CRT were 50 to 150, 10 to 40, and 30 to 130/U/L, 12.0 to 25.8 and 0.4 to 2.3mg/dl respectively (Sharp and la Regina, 1998).

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Table 1: Effects of TMS 98/0505 (Sweet Cassava) leaf ethanolic extract on Biochemical parameters in Albino Rats.

Treatment	Dose Mg/kg	ALT U/L	AST U/L	ALP U/L	BUN Mg/dl	CRT Mg/dl
TMS 98/0505 (Sweet Cassava)	100mg/kg	23.01± 4.73*	103.38± 15.45	81.03± 16.27*	18.03± 4.44	0. 0.04*
TMS 98/0505 (Sweet Cassava)	200mg/kg	21.85± 6.01*	102.05± 16.23	80.05± 16.58*	18.00± 3.62	0.51± 0.03*
TMS 98/0505 (Sweet Cassava)	400mg/kg	24.08± 7.34	104.23± 18.74	84.72± 16.77	18.18± 3.78	0.66± 0.05
Levamisole	7.5mg/kg	19.86± 4.27*	98.80± 19.58	78.61± 17.32*	17.82± 4.62	0.48± 0.04*
Untreated	(control)	29.28± 7.62	120.21± 21.32	102.46± 16.28	19.16± 5.36	0.91± 0.06

KEY:

* = Significant P<0.05 when compared with control
ALT = Alanine amino transferase,
AST = Aspartate aminotransferase
ALP = Alkaline Phosphatase
BUN = Blood urea Nitrogen
CRT = Creatinine

Table 2: Effects of TME 419 (Sweet Cassava) leaf ethanolic extract on Biochemical parameters in Albino Rats.

Treatment	Dose Mg/kg	ALT U/L	AST U/L	ALP U/L	BUN Mg/dl	CRT Mg/dl
TME 419 (Sweet Cassava)	100mg/kg	23.13± 5.03*	105.80±16.32	81.34± 17.55*	18.12±5.22	0.57±0.04*
TME 419 (Sweet Cassava)	200mg/kg	22.01± 4.72*	104.21±15.93	80.28± 16.39*	18.06±4.61	0.55±0.05*
TME 419 (Sweet Cassava)	400mg/kg	24.22± 5.90	106.69±17.08	85.11± 18.17	18.24±3.86	0.71±0.07
Levamisole	7.5mg/kg	19.94± 4.21*	98.72±14.11	78.83± 18.38*	17.84±4.31	0.47±0.05*
Untreated	(Control)	29.29± 6.75	120.27±21.22	102.54± 19.18	19.17±5.29	0.93±0.07

KEY:

* = Significant P<0.05 when compared with control
ALT = Alanine amino transferase,
AST = Aspartate aminotransferase
ALP = Alkaline Phosphatase
BUN = Blood urea Nitrogen
CRT = Creatinine

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

Table 3: Effects of TMS 98/0002 (Bitter Cassava) leaf Ethanolic extract on Biochemical parameters in Albino Rats.

Treatment	Dose Mg/kg	ALT U/L	AST U/L	ALP U/L	BUN Mg/dl	CRT Mg/dl
TMS 98/0002 (Bitter Cassava)	100mg/kg	33.37±5.22	133.56.± 17.13	119.15± 17.26	20.06± 5.42	1.03±0.08
TMS 98/0002 (Bitter Cassava)	200mg/kg	34.28±4.31	135.27± 16.54	120.41± 16.31	20.15± 5.13	1.10±0.06
TMS 98/0002 (Bitter Cassava)	400mg/kg	35.44±5.07*	136.65± 15.21	124.13± 16.02*	20.26± 4.12	1.26±0.05*
Levamisole	7.5mg/kg	19.83±4.28*	98.92± 17.11	78.62± 17.11*	17.84± 4.07	0.46±0.06*
Untreated	(Control)	29.21±7.62	120.23± 22.30	102.58± 16.27	19.15± 5.34	0.90±0.04

KEY:

* = Significant P<0.05 when compared with control
ALT = Alanine amino transferase,
AST = Aspartate aminotransferase
ALP = Alkaline Phosphatase
BUN = Blood urea Nitrogen
CRT = Creatinine

Table 4: Effects of NR/8082 (Bitter Cassava) leaf Ethanolic extract on Biochemical parameters in Albino Rats.

Treatment	Dose Mg/kg	ALT U/L	AST U/L	ALP U/L	BUN Mg/dl	CRT Mg/dl
NR/8082 (Bitter Cassava)	100mg/kg	33.57± 5.23	135.42± 16.19	121.25±17.69	20.13±4.27	1.07±0.07
NR/8082 (Bitter Cassava)	200mg/kg	34.49± 5.32	136.58± 15.82	122.18±16.82	20.22±5.12	1.14±0.06
NR/8082 (Bitter Cassava)	400mg/kg	35.58± 6.08*	138.01± 16.42	124.40±17.38*	20.30±4.26	1.30±0.09*
Levamisole	7.5mg/kg	19.87± 4.33*	98.99± 17.22	78.70±16.91*	17.84±4.31	0.46±0.06*
Untreated	(Control)	29.25± 7.12	120.22± 22.03	102.55±16.25	19.16±5.25	0.92±0.05

KEY:

* = Significant P<0.05 when compared with control
ALT = Alanine amino transferase,
AST = Aspartate aminotransferase
ALP = Alkaline Phosphatase
BUN = Blood urea Nitrogen
CRT = Creatinine

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DISCUSSION

The measurement of some biochemical parameters such as the activities of enzymes in tissues and body fluids play a major role in disease investigation, diagnosis and liver toxicity (Malomo, 2000).

The results obtained from this work revealed a significant ($P<0.05$) dose dependent decrease in values of ALT and ALP at 100 and 200mg/kg in all animal groups treated with leaf extracts of TMS 98/0505 and TME 419 (sweet cassava). This suggests that the species did not have any adverse effect on the liver of the tested animals. Rather they exhibited a hepato-protective effect on the liver of the animals. This concurs with the findings in the work done by Miran *et al.*, (2014) who reported that treatment with cassava leaf flour (CLF) extract inhibited the damage induced by carbon tetrachloride (CCL_4) a hepato-toxin. Mahesh *et al.,* (2010) also studied the hepato-protective effect of scopoletin, against the damage done by carbon tetrachloride (CCL_4), and results showed that the tested group effectively protected the liver of the animals.

Therefore, the present hepato-protective effect of the sweet cassava leaf extract could be as a result of the scopoletin in the leaf, an antioxidant that prevented the accumulation of excessive free radicals.

The significant increase observed in ALT and ALP values in of all animal groups treated with leaf extracts of TMS 90/0002 and NR/8082 (Bitter cassava varieties) at 400mm/kg indicates that the species were not able to protect the liver form damage induced by intestinal parasite infections. There were no significant changes in the AST values in all the animal groups tested.

The ALT enzyme is found in serum and organ tissues, especially liver. An increase in concentration indicates that the extract caused mild changes in the liver. Alkaline phosphate (ALP) is made mostly in the liver and bone with some produced in the intestines. A perforated or punctured intestine can increase levels of the enzyme in the blood.

Levamisole, the commercial anti-parasitc, significantly ($P<0.05$) decreased the values of ALT and ALP. This could be attributed to the therapeutic effect of the medicine which eliminated intestinal parasites in the animals.

There were no significant ($P>0.05$) changes in the BUN values in all the animal groups tested. Treatment with sweet cassava leaf extract (TMS 98/0505 and TME 419) caused a dose dependent decrease in CRT values at 100 and 200mg/kg when compared with the control. This indicates that the species were able to protect the animals from kidney damage. This agrees with the work done by Normasiri *et al.,* (2017) where *Manihot esculenta, crantz* leaf extract repaired the kidneys of rats damaged by gentamycin-induced nephrotoxicity. Sindhu and Kuttan, (2013) also worked on the protective effect of carotenoid letein against cisplatin-induced acute renal failure. The results showed that the carotenoid-lutein, effectively protected the kidneys of mice treated with cisplatin.

Therefore, the nephro-protective effect of the sweet cassava species could be attributed to the carotenoid content of the species.

The significant increase observed in CRT values in all animal groups treated with extracts of bitter cassava leaf varieties (TMS 98/0002, NR/ 8082) at 400mg/kg indicates that the species were not able to protect the kidneys of the animals from damage caused by intestinal parasite infection.

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

Levamisole the commercial anti-parasitic significantly (P<0.05) decreased the values of CRT in all the animal groups. This could be attributed to the recovery of the animals after treatment.

CONCLUSION

In conclusion, this study has shown that sweet cassava leaf species (TMS 98/0505 and TME 419) posses hepato-protective and nephro-protective abilities. They positively influenced the biochemical values of Albino Rats. These medicinal plants are potentially useful phyto-medicines for controlling and maintaining biochemical effects impaired by the intestinal parasites.

On the other hand, the bitter cassava leaf varieties (TMS 98/0002 and NR/8082) negatively influenced the biochemical values of Albino rats infected with intestinal parasites.

Therefore, further experimental studies are required to explore the exact mechanism of actions and extract the active ingredient to be used for the next level of clinical trials to generate novel drugs. This might be helpful to use its immense therapeutic efficacy and ameliorative ability as a potent phytomedicine.

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Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078