Bangl. J. Vet. Med. (2016). 14 (1): 99-102 ISSN: 1729-7893 (Print), 2308-0922 (Online)

PHYTOCHEMISTRY, TOXICITY AND EFFICACY OF CRUDE AQUEOUS EXTRACT OF *CARICA PAPAYA* LEAF AGAINST *TRYPANOSOMA BRUCEI*

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ABSTRACT

The aqueous extract of *Carica papaya* leaf was evaluated for its phytochemistry, acute toxicity and *in vitro* antitrypanosomal efficacy in this study. A total of 15 albino rats grouped into 5(A-E) of 3 rats each were intraperitoneally treated with graded doses of 100, 200, 400, 800mg/kg body weight of the aqueous extract and observed for 24 hours for clinical signs and death. The calculated median lethal dose (LD_{50}) was 600mg/kg body weight, with clinical signs of sluggishness, awkward pasture, loss of appetite, starry hair coat and terminal death within 24 hours. Severity of clinical signs varied with increasing doses. The *in vitro* antitrypanosomal efficacy of the aqueous extract showed 100% inhibition of *Trypanosoma brucei* at concentrations of 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml respectively. In conclusion the aqueous extract of *C. papaya* leaf has bioactive components that are moderately toxic and has trypanosomal inhibitory activity.

Keywords: *Carica papaya*, phytochemistry, acute toxicity, *in vitro* efficacy, *Trypanosoma brucei*

INTRODUCTION

African trypanosomiasis, also known as African sleeping sickness is one of the neglected diseases in about thirty-six countries of sub-Saharan Africa threatening about 40 million human lives (Tyler and Steverding, 2005). *Trypanosoma brucei*, a haemo-protozoan parasite transmitted by tsetse fly causes the disease (Moore, 2005). There are about 23 species of tsetse in sub-Saharan Africa primarily *Glossina morsitans, G. palpalis, G. fusca*. In Nigeria, animal trypanosomiasis is widely distributed from mangrove forest to Sudan savannah due to presence of the tsetse flies in this area (Rocha *et al.*, 2004; Moore, 2005). The economic importance of the disease includes high morbidity, mortality, lower efficiency and high cost of treatment (Brun *et al.*, 2010). The current chemotherapy in humans and animals relies on suramin, pentamidine, melasoprol, eflorithine and diminazene aceturate (Sofowora, 1993). It has been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes (Hoet *et al.*, 2004).

Carica papaya belongs to the family *Caricacea,* and several species of *Caricaceae* have been used as remedy against a variety of diseases (Mello *et al*., 2008). Derived from the southern part of Mexico, *Carica papaya* is a perennial plant and distributed over the whole tropical area. Many scientific investigations have been conducted to evaluate the biological activities of various parts of *C. papaya* including its fruits, shoots, leaves, rinds, seeds, roots or latex (Munox *et al*., 2000). The leaf of *C. papaya* has been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level. This study was conducted to evaluate the *in vitro* trypanosomal inhibitory activity of *C. papaya.*

MATERIALS AND METHODS

Fresh leaves of *C. papaya* were collected within University of Maiduguri Campus in Nigeria and authenticated by a botanist in the Faculty of Science, University of Maiduguri, Nigeria. The fresh leaves of *C. papaya* were rinsed in clean water and air dried in the laboratory for 1 week at room temperature, ground into fine powder using pestle and mortar and sieved to remove debris and coarse plant materials.

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A 1000 gram pre-extraction weight was obtained, which was soaked in 3litres of distilled water, shaken at regular intervals and then filtered using Whatman's number 1 paper. The filtrate was evaporated at 35-40^{o}C in a water bath, allowed to dry on a hot plate and ground using pestle and mortar to obtain a 74.69g powder used for the study. Tests were carried out for tannins, alkaloids, saponins, steroids, phlabotannins, glycosides, flavonoids, free anthraquinones, reducing sugars and ketones as described by Trease and Evans, (2002). Fifteen albino rats obtained from the animal breeding house, University of Maiduguri were used for the study. The rats were kept in a well-ventilated Laboratory and were fed with a commercial poultry feed and given water *ad libitum*. *Trypanosoma brucei* was obtained from Nigeria Institute of Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The Trypanosome was passaged in donor rats before infection of the experimental rats, intraperitoneally with 0.1ml of saline diluted blood containing 1.5 x 10^6 trypanosomes. The level of parasitaemia was determined using the rapid matching method of Herbert and Lumsden (1976). Fifteen albino rats were grouped into 5 (A-E) of 3 rats each and weighed in kilogram (kg) and marked with an ink blotter for easy identification. The rats were intraperitoneally treated with graded doses of 100, 200, 400 and 800mg/kg of the extract based on method of Karber as modified by Aliu and Nwude (1982). The concentration used was 100mg/ml. The rats were observed within 24 hrs for clinical signs. The LD_{50} of *Carica papaya* was then calculated using the modified arithmetic method (Aliu and Nwude, 1982). A serial dilution of the extract (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078mg/ml) was prepared in a phosphate buffered saline solution (PBGS) in different test tubes. Two drops of blood containing the parasite (2 x 10^6) was added into each test tube and incubated at 37^{o}C. The number of motile parasites was counted under the light microscope using improved Neubauer's chamber at 10, 30, 60, 90 and 120 minutes post incubation. The percentage inhibition of motility of the parasite was calculated using this formula below:

% inhibition = (Parasite count of control – Parasite count of treated / Parasite count of control) x 100

Statistical analysis

Inhibition of motility values were expressed as mean ± standard deviation (SD) and percentages.

RESULTS AND DISCUSSION

Flavonoids, alkaloids, steroids, monosacharides, reducing sugars and phlabotannins, free anthraquinones and glycosides were present but saponins was not detected (Table 1).

Table 1. Phytochemical components of crude aqueous extract of *Carica papaya* leaf

Component	Test	Observation	Scoring
Alkaloids	Dragendorff's	Brownish precipitate	+
Flavonoids	Pew's	Red colour	+
Anthraquinones	Borntrager's	Violet colour	+
Steroids	Acetic anhydride	Green colour	+
Saponins	Frothing	Persistent foaming	-
Reducing sugar	Fehling's	Brick red precipitate	+
Phlabotannins	Hydrogen chloride	Red precipitate	+
Monosaccharide	Barfoed's	Red precipitate	+
Glycosides	Salkowski's	Reddish brown colour	+

+ = detected - = not detected

The dose of the extracts that produced 100% mortality of treated albino rats was 800 mg/kg. There were dose dependent clinical signs of sluggishness, awkward pasture, loss of appetite, starry hair coat and terminal death within 24 hours. At doses of 100, 200 and 400mg/kg, mortality was not recorded (Table 2). There was significant ($p<0.05$) decline in mean count ($x10^6$) of *T. brucei* with increase in extract concentration when compared with the normal control. Table 4 shows the *in vitro* percentage inhibition of *T. brucei* by crude aqueous extract of *Carica papaya* leaf. There was 100% inhibition of motility of *T. brucei* at the extract concentration of 2.5mg/ml between 60 and 120 post inoculations; meanwhile the various extract concentrations generally had a positive correlation with inhibition of motility of *T. brucei.*

Table 2. Lethal Dose (ld_{50}) of crude aqueous extract of *Carica papaya* leaf in albino rats

Group (N=3)	Plant Extract (mg/Kg Body Weight)	Dose Difference (DD)	Number Dead	Mean Dead (MD)	DD x MD
A	100		0		
		B – A = 100		0	0
B	200		0		
		C –B = 200		0	0
C	400		0		
		D – C = 400		1.5	600
D	800		3		
Total					600

$LD_{50} = LD_{100} – (DD \times MD/n) = 800 – (600/3) = 600$mg/kg weight

Table 3. *In vitro* efficacy of crude aqueous extract of *Carica papaya* leaf against *Trypanosoma brucei*

Concentration of Extract (mg/ml)	Parasite Count Minutes Post Inoculation (MPI) ($x10^6$)			
	30 mins	60 mins	90mins	120 mins
PBGS (Control)	1.89 ± 0.06 (1.85- 1.83)	1.94 ± 0.09 (2.05 – 1.85)	2.16 ± 0.21 (2.45 – 1.95)	2.14 ± 0.15 (2.35 – 2.05)
0.078	0.61 ± 0.04 (0.65 – 0.75)	0.55 ± 0.08 (0.63 – 0.46)	0.58 ± 0.02 (0.61 – 0.57)	0.52 ± 0.02 (0.55 – 0.50)
0.156	0.51 ± 0.02 (0.53 –0.49)	0.46 ± 0.03 (0.49 – 0.43)	0.36 ± 0.02 (0.38 –0.35)	0.31 ± 0.02 (0.34 –0.30)
0.313	0.40 ± 0.05 (0.4– 0.35)	0.33 ± 0.04 (0.38 – 0.30)	0.27 ± 0.01 (0.29 –0.26)	0.22 ± 0.01 (0.23 – 0.21)
0.625	0.36 ± 0.03 (0.3- 0.31)	0.26 ± 0.03 (0.29- 0.23)	0.19 ± 0.04 (022- 0.14)	0.16 ± 0.04 (0.21- 0.13)
1.250	0.18 ± 0.02 (0.2- 0.16)	0.13 ± 0.02 (0.16 - 0.11)	0.06 ± 0.03 (0.09- 0.02)	0.03 ± 0.01 (0.4- 0.01)
2.5	0.02 ± 0.02 (0.05- 0.01)	0.00 ± 0.00 (0.00 ± 0.00)	0.00 ± 0.00 (0.00 ± 0.00	0.00 ± 0.00 (0.00 ± 0.00
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 4. *In vitro* inhibition rate of *Trypanosoma brucei* by crude aqueous extract of *Carica papaya* leaf

Concentration of Extract (mg/ml)	% Inhibition Minutes Post Inoculation (Mean ± SD)			
	30 mins	60 mins	90mins	120 mins
PBGS (Control)	00 ± 00	00 ± 00	00 ± 00	00 ± 00
0.078	67.66 ± 2.88 (70.77 – 64.87)	71.86 ± 4.49 (77.56 – 68.11)	72.99 ± 2.76 (70.33 – 19.2)	75.50 ± 1.36 (97.74 – 74.15)
0.156	73.26 ± 1.17 (73.86 – 72.13)	76.18 ± 2.26 (79.02 – 73.51)	83.11 ± 1.77 (85.71 – 89.91)	85.49 ± 0.44 (85.53 – 85.00)
0.131	78.68 ± 284 (81.87 – 77.44)	83.35 ± 1.94 (84.88 – 80.51)	87.34 ± 0.71 (88.16 – 86.67)	89.66 ± 0.98 (91.06 – 0.98)
0.625	81.29 ± 1.38 (83.24 – 80.00)	86.0 ± 0.77 (87.577 – 85.85)	90.95 ± 2.23 (94.29 – 89.74)	93.83 ± 2.99 (98.05– 91.06)
1.250	91.82 ± 0.97 (92.97– 90.81)	94.58 ± 0.64 (95.18 – 93.08)	97.23 ± 1.14 (97.98 – 96.10)	98.93 ± 0.48 (99.58 – 98.54)
2.5	99.22 ± 1.22 (100 – 97.44)	100 ± 0.00	100 ± 0.00	100 ± 0.00

PBGS = phosphate buffered glucose solution; MPI = minutes post inoculation.

The phytochemical screening of aqueous extract of *Carica papaya* revealed the presence of flavonoids, alkaloids, steroids, monosaccharides, reducing sugars, phlabotannins, free anthraquinones and glycosides while saponins was not detected. Mello *et al.* (2008) reported similar phytochemical compounds from *C. candamascensis*. The median lethal dose (LD_{50}) of the leaf crude extract was calculated as 600mg/Kg. This falls within the range between 500 to 1000 mg/Kg which has been classified as moderately toxic (Hodge and Sterner, 1949). It has been reported in several studies that toxicity is related to the effects of the extracts on the liver, kidney and other functional organs of animals by altering their specific enzymes (Bruno *et al*., 2013). The *in vitro* efficacy of the extract on *T. brucei* at different concentrations in this study revealed 100% inhibition of motilily at concentrations of 2.5mg/ml and above. This agrees with Sepulveda-Boza and Cassels (1996) that many natural products exhibit their trypanocidal effect by acting either on the respiratory chain or on the cellular defences against oxidative stress. This also confirms with the reports of Bruno *et al*. (2013), which indicated that the trypanocidal activities affects different targets or biologically significant processes in the parasite, generate intra-parasitic toxic radicals through 5-nitrofuryl moiety and inhibition of cruzipain through the thiosemicarbazone pharmacophore. It is also known that some agents act by binding with kinetoplast DNA of trypanosomes (Beiyu *et al*., 2006).

In conclusion, results from this study have shown that crude aqueous extract of *Carica papaya* leaf contained important secondary metabolites, moderately toxic in rats and has *in vitro* trypanosomal inhibitory action.

REFERENCES

1. Aliu YO and Nwude NI (1982). *Veterinary Pharmacology and Toxicology Experiments*. ABU Press, Zaria, Nigeria. pp. 104-110.
2. Beiyu L, Henrik M, Dario K, Akhilesh P and Paul T (2006). Role of p38 in replication of *Trypanosoma brucei* kinetoplast DNA. *Journal of Molecular Cell Biology* 26: 5382.
3. Brun R, Blum J, Chappuis F and Burri C (2010). Human African trypanosomiasis. *Lancet* 379: 148-159.
4. Bruno D, Miriam R, Francesco C, Daniel L and Claudio O (2013). Potential mechanism of the anti-trypanosomal activity of organoruthenium complexes with bioactive thiosemicarbazones. *Biological Trace Element Research* 153: 371-381.
5. Herbert WJ and Lumsden WH (1976). *Trypanosoma brucei* method of estimating the host's parasitemia. *Experimental Parasitology* 40:427-431.
6. Hodge HC and Sterner JH (1949). Toxicity Rating. *American Industrial Hygiene Association Quarterly* 10: 93-96. Doi: 10.1080/00968204909344159.
7. Hoet S, Stevingy C, Block S and Baldeyrov B (2004). Antitrypanosomal activity, cytotoxicity and interaction with DNA and poisomerase. *Plant Medicine* 70: 405-513.
8. Mello VJ, Gomes MT, Lemos FO and Delfino JL (2008). The gastric ulcer protective and healing role of cysteine proteinases from *Carica candamascensis*. *Journal of Phytomedicine* 15: 237-244.
9. Moore AC (2005). Prospects for improving African trypanosomiasis chemotherapy. *Journal of Infectious Diseases* 191: 1793-1795.
10. Munox V, Sauvain M, Bourdy G, Callaba J, Rojas I, Tae A and Deharo E (2000). The search for natural bioactive compounds through a multidisciplinary approach in Bolivia. *Journal of Ethanophamacology* 69: 139-155.
11. Rocha G, Martins A, Gama G and Brandco F (2004). Possible causes of sexual and congenital transmission of sleeping sickness. *Lancet* 363: 247.
12. Sepulveda-Boza S and Cassels BK (1996). Plant metabolite active against *Trypanosoma cruzi. Journal of Medicine* 62: 98-105.
13. Sofowora A (1993). *Medicinal Plants and Traditional Medicine in Africa*. 2nd Ed. Spectrum Books Ltd., Ibadan, Nigeria. pp. 134-156.
14. Tyler KM and Steverding D (2005). Novel Anti-trypanosomal agents. *Expert opinion on Investigational Drugs* 14: 939-955.
15. Trease GE and Evans WC (2002). *Pharmacognosy*. 15th Ed. Saunders. pp. 214-393.