

Curcin from *Jatropha curcas* seed as a potential anthelmintic

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ABSTRACT

The study was done with the aim to evaluate the anthelmintic activity of curcin from *Jatropha curcas* seed using adult *Pheritima posthuma* against Niclosamide (15 mg/ml) as standard references and normal saline as control. The activities of the possible crude curcin was determined on the tested parasites in this study so as to verify if the *J. curcas* seed would be a potential sources of useful anthelmintic drugs by monitoring the time to achieve paralysis of the worms was determined. In the acute toxicity test, toxic signs were observed at 300 mg/kg. However, the *P. posthuma* displayed physical changes with LD₅₀ of 800 mg/kg per body weight. The toxicity of curcin in *P. posthuma* is high; however, caution should be exercised in its use especially at high doses.

Keywords: *Jatropha curcas*, immounotoxins, crude curcin, niclosamide, *Pheretima posthuma* and anthelmintic activity.

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INTRODUCTION

Distributed in many tropical and subtropical countries, *Jatropha curcas* L belongs to the family Euphorbiaceae. All parts of *J. curcas* are considered toxic in particular the seeds; its toxicity has been attributed to a protein component. This toxic protein was isolated from the seeds of *J. curcas* by Felke (1914), and was designated as "curcin". He proposed that the curcin was a kind of toxalbumin (Felke, 1914). Many plants contain proteins that are capable of inactivating ribosome and accordingly are called ribosome-inactivating protein (RIP). RIP are usually divided in 2 subgroups on the basis of their structure and functions: Type I RIP consisting of a single polypeptide chain with Mr 28 000-35 000 and alkaline isoelectric points (pI) of pH 8 to 10 with or without carbohydrates; type II RIP consisting of a catalytically active A chain linked to a cell-binding B chain. The A chain is the functional equivalent of a Type I RIP, and the B chain is a lectin (Barbieri et al., 1993). Barbieri et al. (1993) reported that the curcin is type I ribosome inactivating protein. Curcina purgative oil and a phytotoxin or toxalbumin (curcin) similar to ricin in Ricinis.

Ribosome-inactivating proteins (RIPs) from plant are toxins that can inhibit protein synthesis in eukaryotic cells by catalytically damaging ribosomes (Barbieri et al., 1993). With rRNA N-glycoside activity, when entering a cell the protein can enzymatically cleave the specific glycosidic bond of an adenine A₄₂₃₄ of 28S rRNA, thus inhibiting protein synthesis by interfering with the binding of the 28SRNA to elongation factors (Peumnas et al., 2001).

RIPs are believed to be involved in plant defence systems and have anti-fungal and anti-viral activities (Krawetz and Boston, 2000) as well as anti-tumour activity (Lin et al., 2003). In addition RIPs are of great interest in theory and practice (Shaw et al., 1991). They have been used as a mid-term aborting agent (Cheung et al., 1989) and for the treatment of hydration moles (Lu and Jin, 1990).

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and

cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia (Bundy, 1994). Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas (Tagbota and Townson, 2001). The major aim of the study is to evaluate the medicinal potentials of *J. curcas* with special attention on their anthelmintic potentials.

MATERIALS AND METHODS

Collection of plant

About 1000 g of *J. curcas* seeds were collected from a matured tree from a local farm land in Nasarawa State, Nigeria.

Extraction and isolation of Curcin

The seeds were dried at ambient temperature and pulverized into coarse powder in a grinder machine. 200 g of dried seed powder was extracted with 2000 dm³ of hexane (BDH Chemicals Ltd, England) by cold maceration process. Solvent from the sample was filtered, squeezed off by Buchner funnel connected to a vacuum pump (XZ-1B vacuum pump) and evaporated off under reduce pressure in a rotary evaporator (Popular India; model: PT-69, serial number: 513) to obtain crude extract.

The resulting dried crude extract is extracted with 1 L of cold 0.005 M-sodium phosphate buffer, pH 7.2, containing 0.2 M-NaCl for 24 h. Solid ammonium sulphate was added to the supernatant to about 60% saturation. After being left overnight, the precipitate was collected by centrifugation at 1200 rev/min for 20 min, and then re-dissolved in 0.005 M sodium phosphate. The solution was dialysed with the same buffer. At the end of dialysis, a brown precipitate was present in the bags, and then removed out by centrifugation for anthelmintic investigation (Stirpe et al., 1976). The brownish supernatant is referred to as crude curcin.

Drugs and chemicals

15 mg/ml of Niclosamide (Micromedex) and Normal saline were used as reference standard and control.

Experimental animal

Adult earthworms (*P. posthuma*) having anatomical and physiological resemblance with intestinal roundworm parasite of the human being were used for present study (Chatterjee, 1967). *P. posthuma*, was collected from moist soil, and washed out in to normal saline water and the earthworms are divided into five groups. Each groups consisting of fifteen earthworms (approximately equal size).

Anthelmintic investigation

According to procedure Nirmal et al. (2007) and Ravindra et al. (2007); five groups of fifteen earthworms in each group with approximately equal sized were released into 50 ml of desired solution. Each group was treated with normal saline (control), Niclosamide (15 mg/ml), and crude curcin (10, 25 and 50 mg/ml). Observations were made for the time of paralysis of individual worms. Paralysis assumed to occur when the worms did not revive

even in normal saline.

Acute toxicity test

The method was performed according to WHO guideline (WHO 2000) and the OECD guideline for testing of chemicals 420 (OECD 2001). The earthworms were randomly divided into five groups of ten. Adult earthworms, with approximately equal sized, were selected for testing. Earthworm was washed briefly with deionized water, and an earthworm was introduced per vial and the vial was covered with plastic film that had been punched with small holes using needles. The animals were then observed for mortality at higher doses such as 200, 300, 400, 800, 1600 mg/kg body weight. Tests were done in the dark at ambient temperature for 24 h. After 24 h the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part.

RESULTS AND DISCUSSION

The anthelmintic effect of crude curcin extract is shown in Table 1 and the extract showed dose dependent activity. 10 mg/ml of extract paralysed the experimental group within 13 min and death in 34 min, 20 mg/ml paralysed the experimental group 10 min and death in 25 min; while at 50 mg/ml paralysis was observed at 4 min and death at 15 min. The result shows that the anthelmintic activity of crude curcin is more potent than the presently used Niclosamide. Crude curcin intense activity might be attributed to its use as components of 'immunotoxins', a type of hybrid molecules consisting of a toxic peptide chain linked to an antibody (Frankel et al., 1986). However, there are some problems in the application of immunotoxins, such as poor stability, immunogenicity and promotion of vascular leak syndrome, which would raise serious questions on their application (Kreitman et al., 1999). Secondly, literature reports by Ahirrao et al. (2011), revealed that *J. curcas* are rich in alkaloids, and saponins which act by diminishing the support of glucose to the helminthes, acts on CNS causing paralysis (Sutar et al., 2010; Mute, 2009; Sharma et al., 2010; Mali et al., 2007) and enhance intestinal absorption of Na⁺ and water (Kumar et al., 2010) respectively, in the crude curcin known to possess anthelmintic activity.

In the acute toxicity study (Table 2), all the earthworms died within the 24 h study period at a concentration of 1600 mg/kg body weight. Earthworms treated with 200 mg/kg of crude curcin exhibit some signs of adverse effects such as, the earthworm displayed change of skin colour and reduced mobility. However, adverse signs, mainly cholinergic in nature, were observed at doses above 300 mg/kg of the extract. From the data obtained in this study LD₅₀ of the extract was found to be 800 mg/kg body weight. This strongly suggests that crude curcin has high toxicity since acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at very low doses. Hence multiple dose studies are almost always invaluable in evaluating the safety profile of phytomedicines.

Table 1. Anthelmintic activity of crude curcumin extract.

Group	Treatment	Conc. (mg/ml)	<i>Pheretima posthuma</i> (Earthworm)	
			Time taken for paralysis (min)	Time taken for death (min)
I	Normal saline		-	-
III	Niclosamide	15	23 ± 1.15	61 ± 0.88
III		10	13 ± 0.41	34 ± 1.20
IV	Crude curcumin	20	10 ± 0.46	25 ± 1.18
V		50	04 ± 0.58	15 ± 1.36

Table 2. Acute toxicity test after administration of crude curcumin in *Pheretima posthuma*.

Dose (mg/kg)	% Mortality
200	0
300	30
400	30
800	60
1600	100

CONCLUSION

This effect may be explored in the possible use of the curcumin as an anthelmintic agent. Due to the toxicity of curcumin to man and domestic animals further pharmacological and biochemical investigation to look for new RIP to identify those with the less immunotoxins, activity, to select the most suitable ones for human therapy and to overcome the immune response that follows clinically oriented administration of RIP conjugates. Toxicity of the extract in earthworms is high. However, since this finding cannot be directly extrapolated to humans, caution should be exercised in its use especially at high doses.

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