

Histological changes in the liver following use of *Brassica oleracea* extract as an antitrypanosomal agent in Sprague-Dawley rats

R.E. UCHEYA¹, J.C. IGWEH², U.S.B. ANYAEHIE², E.O. NWOSU¹ and M.L. NWEKE²

¹Department of Anatomy, ²Department of Physiology,
College of Medicine, University of Nigeria, Enugu campus (Nigeria)

(Received: July 18, 2007; Accepted: August 28, 2007)

ABSTRACT

In the present investigation, we studied the changes in the liver of Trypanosomal infected Sprague dawley rats that were treated with extracts of *Brassica oleracea*. Thirty Sprague-Dawley rats divided randomly six equal experimental groups with two serving as control groups were used. Rats into four groups were infected with a strain of *T. brucei* and subsequently treated with either oral or intramuscular injections of methanolic and aqueous extracts of *Brassica oleracea*. The control groups consisted of a group of rats infected but not treated and another group that were uninfected but treated with intramuscular injection of aqueous extracts. The aim was to determine the safety and also substantiate reported anti-trypanosomal activities of *Brassica oleracea*.

Our findings showed that both methanolic and aqueous extracts of *Brassica oleracea* attenuate liver damage due to Trypanosomiasis infection thereby prolonging life of infected rats. This confirms *Brassica oleracea* as safe but with limited anti-trypanosomal activity. We thus recommend extracts of *Brassica oleracea* as a suppressant of Trypanosomiasis which can be a useful adjunct to therapy.

Key words: *Brassica oleracea*, liver, Trypanosomes, Sprague-dawley rats.

INTRODUCTION

Medicinal plants are widely used by poor populations all over the world (Ranos, 2006) and have been integrated into health scheme in some countries despite advances in orthodox medicine (Nwangwa *et al*, 2007). Trypanosomiasis is widely distributed in Africa and the existing curative drugs are not only short in supply but also expensive (Molyneux, 1997). *Brassica oleracea* also known as wild cabbage, which was initially grown along coasts of Europe and North America, but now grown worldwide is enjoying increasing recognition as a medicinal plant. The Romans for instance ate cabbage to cure hangovers and now the American cancer society recommends increase of intake of cabbage and other crucifer crops (Salathia *et al*,

2007). Aqueous extracts of *B. oleracea* according to Igweh *et al*, 2002 demonstrated in-vitro anti-trypanosomal activities as evident from inability to immobilize strains of *T. brucei* and subsequent finding that the immobilized trypanosomes were unable to infect mice. Other reports have documented medicinal properties of *B. oleracea* as an anti-fungal agent (Sistu *et al*, 2003) and lately antioxidant (Okazaki *et al*, 2007) with promising anticancer activity (Salathia *et al*, 2007). The possibility of its anti-trypanosomal activity in addition to documented anti-carcinogenic property is boosted by the fact that protozoan parasites have a number of common features with the proliferating cancer cells such that anti-tumor drugs are also screened for possible anti-trypanosomal action (Caciunescu *et al*, 1993).

This study aims to investigate the in-vivo anti-trypanosomal activity of aqueous and methanolic extracts of *Brassica Oleracea*, as well as its safety using histological changes in the liver.

MATERIAL AND METHODS

Plant material

Aqueous and methanolic extracts from Fresh *Brassica Oleracea* leaves collected in January, 2006 from Enugu, South-Eastern Nigeria and authenticated at the Botany Department of University of Nigeria, Nsukka were used in this study. Extraction and isolation were as described below

1. *Aqueous extract*: 50grams of dried leaf extracts was boiled in 3750 of distilled water for five hours. The filtrate was oven dried at 600 °C after evaporation to yield brown colored dry extract.
2. *Methanolic extract*: 50 grams of crushed dry leaves was soaked in 200mls of methanol and left in a closed aluminum container for seven days. The filtrate was subsequently distilled and dried by evaporation to yield a deep-greenish extract.

Animals

30 Sprague Dawley rats divided into six equal groups (Groups 1-6) and acclimatized for 2 weeks were used. They all had water and pelleted food ad libitum. All procedures and care of the

animals conformed to International guideline, principle of laboratory animal care. Animals that were alive at the end of the experiment were sacrificed by cervical dislocation.

Trypanosomes

Strains of *T. brucei* isolated from cattle in Federe, Northern Nigeria by The Nigerian Institute of trypanosomal Research, Vom, Jos.

Infection of Rats

Blood obtained from the tail vein of infected animals was diluted with 0.85% physiological saline. then 0.1ml was injected intra-peritoneally into the twenty rats in groups 1-5. Daily microscopic examination of wet mounts preparation of blood from the rats confirmed infection by the 2nd day post intra-peritoneal injection of the *T. brucei* strain.

Rats in group 6 were not infected and served as a set control (C2).

Administration of extracts

Groups 1 and 2 (M1 and M2) had Methanolic extracts. M1 had 50mg as intramuscular injections while M2 received 100mg orally. Groups 3 and 4 (A1 and A2) had aqueous extracts. A1 received 50mg as intramuscular injection while A2 had 100mg orally. Group 5 (C1) served as control and were not treated. Group 6 (C2) received 100mg of aqueous extract as IM injection.

Table 1: Animal Grouping and experimental findings

Animal groups	Remarks	Fate
1. M1	Infected, then given 50mg of methanol extract intramuscularly 2days after.	Died on the 4 th day post infection.
2. M2	Infected, then given 100mg of Methanolic extract orally	Died on the 6 th day post-infection
3. A1	Infected, then given 50mg aqueous extract intramuscularly	Died on the 8 th day post-infection
4. A2	Infected, then given 100mg aqueous extract orally	Died between the 6 th -8 th day post-infection
5. C1	Infected, but not treated	Died on the 3 rd day post infection
6. C2	Not infected, but given 100mg injection of aquoes extract	Sacrificed by cervical dislocation on death of others.

RESULTS

Results presented as Table 1 and 2 show the following:

H/E stained sections of the Liver of all infected rats (Table 2) shows trypanosomal-induced pathological changes in the Liver. The pathological changes were minimal in the treated groups 1-4 compared to the untreated infected group 5. Table 1 shows that the treated rats also had an extended life-time of between 6-8 days post-infection compared to 3 days in the untreated group 5 (C1). Also the uninfected but treated group 6 rats (C2) were all alive 2 days after death of all other rats. Hepatic histological finding after being sacrificed showed a normal liver architecture.

DISCUSSION

Trypanosomiasis affects both man and livestock and this research documents a high mortality due to the infection with the death of all

Trypanosomal infected Sprague Dawley after 3 days in the absence of treatment (Table 1). Igweh *et al.*, 2002 documented in-vitro anti-trypanosomal activity by extracts of Brassica Oleracea, a view supported by our finding that treatment with extracts of *B. oleracea* extended the life of infected rats up to a range of 6-8 days. This extended life however supports limited anti-trypanosomal activity by *B. oleracea* which appears suppressive rather than earlier report of cure. The eventual death and presence of Trypanosomes in the vascular lumen of even the treated groups supports our view of suppressive anti-trypanosomal activity.

The pathological changes due to untreated Trypanosomiasis are documented by microscopic finding in the stained sections of the liver from C1 group (Table 2). These changes as seen in table 2 were minimal in the treated groups. This is suggestive of ability of the extracts to either immobilize the trypanosomes in-vivo or offer some hepatic cyto-protective property. This is possibly due

Table 2: Histological Findings

Animal groups	Trypanosomes in vessels	Histological findings			
		Portal Hypertension	Venous congestion	Eosinophilia	Other findings
M1	++	+	++	++	Peri-nuclear holes
M2	+	++	+	+	Nuclear hypochromasi
A1	++	+	+	Nil	Nil
A2	+	+		+	Paranchymal hyalination
C1	+++	+++	+	+	Necrotic and pyknotic areas
C2	nil	nil	nil	nil	Normal archetecture

+ = Severity

to documented anti-oxidant properties of *B. oleracea* (Okazaki *et al.*, 2007). There is also the possibility that the anti-trypanosomal activity of Brassica Oleracea which extended the life of the animals may have limited the injury to the liver. However, the presence of Trypanosomes in the vessels of these treated rats supports the anti-oxidant theory rather than anti-trypanosomal property.

The control group of rats that received the extracts in absence of induced infection with trypanosomes showed no evidence of liver cell damage (Table 2). This agrees with earlier studies which are suggestive of the safety of *B. oleracea* and also supports the assertion that natural products in correct forms and dosage are less harmful than synthetic products (Olatunji, 2005). The injection of

aqueous extract had an overall suppressive effect than other routes/doses and even a double dose in the control group was safe. There is also a possibility that the anti-trypanosomal activities of *B. oleracea* extracts may be dose dependent such that an increased dosage may actually be curative.

Conclusion

Extracts of *B. oleracea* when used as an anti-trypanosomal agent attenuates parasite-induced liver damage most likely due to its anti-oxidant properties. It is thus safe, but we recommend its use as an adjunct to therapy since suppressive rather than curative effect is reported in this study.

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