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Acute Toxicity and Growth Suppression Effects of the Ethanolic Extract of *Cryptolepis Sanguinolenta* (Lindl) Schletcher

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***Cryptolepis sanguinolenta,* a member of the Asclepiadecae family is used extensively in Africa to treat malaria and several other conditions. This study was done to evaluate the toxicity of the plant in mice and rats. The ethanolic extract of the plant was studied. The extract exhibited LD_{50} of 2.08 g/kg (p=0.05) in mice, with a GS_{50} of 0.44 g/kg (p=0.05) in rats. Comparatively the compound was found to be only 1/5 as toxic as chloroquine, the standard antimalarial drug in Uganda, showing growth suppression 1/10 that of chloroquine. It was concluded that *C.sanguinolenta* is a safe drug that should be further developed for treatment of malaria.**

Key Words: *Cryptolepis sanguinolenta*, Asclepiadecae, malaria, toxicity.

INTRODUCTION

Plants have been used by man as medicines either in their raw forms or as crude extracts for centuries. A medicinal plant is any plant which, in one or more of its organs (roots, and aerial parts), contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Medicinal plants are now screened for bioactive agents and developed into drugs and dosage forms [1,2]. Today, at least 25 per cent of drugs used in Western medicine are derived from plants [3]

Researchers have become aware of the toxicity of African medicinal plants amidst the euphoria of their usefulness. Toxicity tests now represent some 2.5 per cent of the total publications on African medicinal plants [1]. As more plant derived drugs come into use, there is need to carry out toxicity studies to determine acceptable from non-acceptable toxicity levels.

Crytolepis sanguinolenta, a climbing plant which belongs to the Asclepiadaceae family, is widely used throughout tropical Africa, including Uganda. It is used for the treatment of various diseases such as meningitis, diarrhoea, migraine headaches and malaria. Extensive studies on the plant and its extracts have been carried out, but these covered the pharmacological profiles and efficacy studies.

In vitro antibacterial activities against diarrhoeal bacteria such as Vibrio cholera and Campylobacter were demonstrated using ethanolic extracts of the plant [4]. However chloroform and water extracts of the plant were inactive against the same bacteria including Pseudomonas, Klebsiella and E.coli [5].

The antihyperglycaemic activity of the water extract has not only been demonstrated but has also been patented [6,7]. In all these studies it appears no safety profiles of the plant extracts were investigated.

The antimalarial activity of the crude methanolic extract of *C.sanguinolenta* against *P.falciparum* was reported [8]. Antimalarial studies against *P.falciparum* using the semi-pure ethanolic extract of the plant [9], compared very favourably with the ED_{50} of 0.2 µg/ml obtained for chloroquine [10] and it was concluded that it may be a useful drug for malaria.

The continuous medicinal use of *C.sanguinolenta* by the community may have unrecognised toxic manifestations. This study was an attempt to establish the toxicity profile of the semi-pure ethanolic extract of the plant, a further step towards the development of the extract as a therapeutic agent.

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EXPERIMENTAL

Preparation of the extract (solid C)

The aerial parts and roots of the plant was collected from Kumi district (Eastern Uganda) and authenticated by a taxonomist at the Natural Chemotherapeutics Research Laboratory, Kampala. Voucher specimen has been deposited at the herbarium, Department of Pharmacology and Therapeutics, Makerere Medical School. The roots were analysed in the experiments.

The roots were washed under tap water and dried at room temperature. The dried roots were pulverised into a fine powder. The powdered root (100 g weighed with a Metler Electronic balance) was soaked in 500 mL of ether (Ether anaesthetic BP 88, Pharmadrug, Wurmlingen, Germany) and allowed to stand at room temperature for 24 hours. After 24 hours, the ethereal extract was filtered and discarded. The marc was dried and soaked in 500 mL ethanol for 4 days, then filtered, and the marc re-soaked in ethanol. The ethanol extracts were combined and concentrated by low - pressure distillation of the solvent to about 1/3 the original volume. The concentrated ethanol extract was partitioned between ether and ethanol. The ether soluble fraction was discarded. The ethanol soluble portion was diluted with an equal volume of ethanol and allowed to stand in a conical flask. After about 3 days some crystals were formed. The mother liquor was decanted and the crystals washed in cold ethanol and dried to give semi-pure crystals (0.3% yield), which were used in the bioassay. These were labelled solid C.

Preparation of injectable compound C

Compound C was sparingly soluble in water. Injection form was obtained by dissolving it in acidified water by adding drops of 2 % Hydrochloric acid and neutralizing with 0.1M Sodium Hydroxide and buffering with Potassium Orthophosphate/Sodium Hydroxide [11].

Determination of the LD_{50} of solid C in mice

A preliminary test was carried out to determine the approximate LD_{50}. Five pairs of mice, starved over-night, were given varying doses of the extract at 0.3, 1.0, 2.5, 3.5, and 5.0 g/kg orally via an intra-gastric tube. The animals were kept in cages and allowed food and water *ad lib*. The dose that killed one or both mice was regarded as the preliminary median lethal dose.

The acute toxicity value, the Median Lethal Dose (LD_{50}), was determined using a range of doses around the preliminary value in a larger group of mice. Using 36 mice divided into 6 groups, they were given doses of the extract as follow: 1.0, 1.5, 2.0, 3.0, and 3.5 g/kg orally *via* an intra-gastric tube. The control group was given distilled water by the same route. The animals were observed closely during the first two hours, then half hourly for the next four hours, and then six hourly up-to 24 hours. Changes in behaviour, activity, respiration, piloerection, lacrimation, proptosis, photophobia, micturition, and convulsions or death were recorded. The number of mice that died were converted into probits and used to calculate the LD_{50} and the 95% confidence limits.

Another group of 30 mice were given compound C by subcutaneous route in doses ranging from 0.5, 1.0, 1.5, 2.0 and 2.5 g/kg. The control group were given the buffer solution by the same route. They were observed as was done after the oral dosing. After 24 hours the number of dead mice were obtained and used to obtain the LD_{50}.

The 95% confidence limits were calculated using the Litchfield and Wilcoxon method [12], while probit analysis was used to calculate LD_{50}.

Determination of the LD_{50} of chloroquine in mice

The LD_{50} of chloroquine (Chloroquine Phosphate Injectable by Intas Pharmaceuticals Ltd of India) was determined by dosing 5 pairs of mice, in the same manner as was done for solid C. Single doses of chloroquine ranging from 0.1, 0.2, 0.3, 0.5 and 1.0 g/kg were given orally and subcutaneously. The volume of each dose ranged from 0.5 to 1.0 ml orally with an intra-gastric tube, and, 0.1 to 0.5 ml for the subcutaneous route using a tuberculin syringe and needle. The animals were kept in cages and allowed food and water *ad lib*. The LD_{50} of chloroquine was then calculated in a similar manner as above.

Determination of growth suppression of solid C in rats

By using a modified method by Smith [13] and Ghosh [14] sub-lethal (SLD) oral dose in rats was found to be 1.4 g/kg.

Thirty young male rats, aged 21 days, with average weights of 40 g, were divided into 5 groups of 6 rats each. One of the groups was used as the control group and orally given distilled water. The remaining 4 groups were dosed with solid C daily. Using the predetermined SLD, the first group was dosed with 0.375 g/kg, the 2nd group with 0.75 g/kg, the 3rd group with 1.125 g/kg and the 4th group with 1.5 g/kg corresponding to 1/4, 1/2, 3/4 and 1/1 of the SLD, respectively for 14 days. The animals were weighed daily before each dosing and were allowed food and water *ad lib*. On the 14th day, the average weight gain for each group was determined. The percentage growth suppression in the test group was calculated as follow:

Weight (wt) gain = wt_0 - wt_{14} (1)

Where:
wt_0 = the starting average weight
wt_{14} = the average weight on day 14,

wt gain difference = wt gain (C) - wt gain (T) (2)

where C = control group, and T = test group.

(this is the extent of growth suppression expressed as weight not gained by test group)

Therefore % growth suppression = wt gain diff./ wt gain (C) x 100 (3)

The weight gain differences were converted into percentage growth suppression and then plotted as log dose-percentage suppression curve from which the Median Growth Suppression (GS_{50}) was obtained and the 95% confidence limits calculated by the Litchfield and Wilcoxon method.

RESULTS

The LD_{50} of solid C by the oral route was found to be 2.08 (1.73 to 2.5) g/kg at p = 0.05, while the LD_{50} of solid C by the subcutaneous route was found to be 1.74 (1.45 to 2.09) g/kg at p=0.05.

The LD_{50} of chloroquine by oral route with a 95% confidence limits was determined to be 0.45 (0.30 to 0.68) g/kg at p = 0.05. While the LD_{50} of chloroquine by the subcutaneous route was 0.39 (0.30 to 0.51) g/kg at p = 0.05.

Solid C demonstrated growth suppression (GS_{50}) effect value of 0.44 (0.20 to 0.99) g/kg with 95% confidence limit. All rats in the full SLD group died by the 6th day of dosing and all had lost weight by over 30 % by the time of death.

DISCUSSION

C.sanguinolenta has been shown to be much safer than chloroquine in its acute toxicity and with less growth suppression effect.

The extraction process gave a yield of about 0.3 % of the solid C. The characteristic arrangement of the crystals, (deep orange to red central portion surrounded by bright yellow outer zone), and their amorphous nature imply that the compound may not be a pure substance [15]. However the reactions of the compound indicates that it is a basic substance, as demonstrated by its ready solubility in acidic medium and its ready precipitation in alkaline medium. The poor solubility of the compound in water suggests that only small amounts are needed for the beneficial medicinal effects attained by the traditional healers. Both oral and subcutaneous route were used to measure the bioavailability of solid C.

In the use of parenteral solid C, the animals did not show any reactions to the buffered solution. This is because the buffer system used is expected to operate in the body [13]. This was confirmed by the fact that the control group were given the buffered solution at pH 7 with no effect observed. The eventual compounding of the product for parenteral use will involve the use of its salts, like in the case of quinine and chloroquine [16]. In this study, the hydrochloride of the compound was used.

The acute toxicity of oral solid C at LD_{50} = 2.08 g/kg, p = 0.05) was found to be close to that obtained by parenteral solid C at LD_{50} = 1.74 (p = 0.05), this demonstrated that solid C has a good bioavailability by oral route thus permitting oral preparations as the main form of administration of the developed drug. The compound was found to be 4½ times more toxic than the crude extract (LD_{50} = 9.4 g/kg, p = 0.05) obtained by Waako [8]. This may have been so as a result of purification, thereby enhancing the acute toxicity of the semi-pure extract in solid C.

The acute toxicity of solid C compares very favourably with that of chloroquine, with the compound being 1/5 as toxic. Chloroquine is known to have more than 90 % bioavailability. This was confirmed by the study. Chloroquine was found to induce seizures in rats at doses between 0.04 - 0.1 g/kg [17], but solid C did not induce any seizures below 1.5 g/kg. However, convulsions were observed in mice that got more than 1.5 g/kg, just before there death. Therefore the compound, according to the classification by Ghosh [14], would be regarded as only 'slightly toxic' (LD_{50} of 0.5 - 5 g/kg).

Growth suppression is regarded as an effective measure of the toxicity of most drugs. The method used for dose designing in the sub-acute tests is based on the fact that the SLD is the highest tolerable dose (survival of up-to 70%). This however is not applicable to drugs that have growth stimulating or complete growth suppression effect. The inability to gain weight by young male rats was used as a measure of the toxicity solid C. The choice of 14 days duration has been shown to give as good a result as 21 days test [13,14].

A compound usually kills some rats whenever the amount administered is sufficient to cause a consistent reduction of the group mean weight as was noted by the deaths of the animals during the sub-acute toxicity tests. In choosing a compound for further development into a therapeutic agent, the point to be considered is not the presence of toxicity, but rather, the level of the toxicity, and whether the medicinal benefits outweigh its toxicity [18]. At a GS_{50} of 0.44 g/kg (p = 0.05), solid C is still a much safer drug than chloroquine with GS_{50} of 0.048±0.008 g/kg [13]. From simple comparison, the relative toxicity of solid C against chloroquine would be about 1/10 that of chloroquine.

CONCLUSIONS

It can be concluded from this study that solid C has a good safety profile with acute toxicity of only 1/5 and growth suppression effect of 1/10 that of chloroquine. Since solid C has been found to be effective in chloroquine resistant malaria [8], and is safer than chloroquine as shown in this study, with the wide distribution and availability of the plant in Uganda [19], making it readily available, it is therefore recommended that:

a) Further toxicity studies to be carried out on the compound, to determine long-term safety.

b) And that solid C, considered on the basis of the demonstrated toxicity profile in the two species of animals studied, is a safe drug that deserves further development for possible use in the treatment of malaria.

c) This extract can be promoted for use by the traditional healers as a safe therapy for malaria.

d) Phase I clinical trails are recommended.

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REFERENCES

[1] A. Sofowora. Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Limited, Ibadan - Oweri - Kaduna - Lagos. (1993) pp.6, 159-176, 179-189, 195-238.

[2] M. Wichtl. Quality Control and Efficacy Evaluation of Phytopharmaceuticals, Plants for food and Medicine, Miscellaneous Contributed Sessions, 2-3 July, 1996. Imperial College, London, UK. 40-41.

[3] S. Gunnar. Bull. Chem. Soc. Ethiop. 1 (1987) 47-54.

[4]. A. Paulo, M. Pimentel, S. Viegas, I. Pires, A. Duarte, J. Cabrita, and E.T. Gomes. J. Ethnopharmacol. 44 (1994) 73-77.

[5]. A. Paulo, A. Duarte, and E.T.Gomes J. Ethnopharmacol 44 (1994) 127-130.

[6]. D.E. Bierer. Hypoglycemic agent from cryptolepis, PATENT-US - 5,753,790: 20PP

[7]. J. Luo, D.M. Fort, T.J. Carlson, B.K. Noamesi, D. Nii-Amon-Kotei, S.R. King, J. Tsai, J. Quan, C. Hobensack, P. Lapresca, N. Waldeck, C.D. Mendez, S.D. Joland, D.E. Bierer, G.M. Reaven, Diabetic Med 15 (1998) 367-374.

[8] P. Waako. A Study of the Antimalarial and Antibacterial Activity of the Crude Root Extracts of *Secamone africana*. M.Sc. THESIS, Makerere University (1996).

[9] J.W. Ogwal-Okeng. Studies on the Antimalarial Activites of some Uganda Medicinal Plants. Ph.D. Thesis, Makerere University (1998).

[10] K.H. Rieckman, G.H. Campbell, L.J. Sax, and J.E. Mrema. The Lancet, 7 (1978) 22 23.

[11] E. Owino. -Personal communication, Dept. of Pharmacy, Makerere University (1999).

[12] J.T. Litchfield and F. Wilcoxon. J. Pharmacol. Exp. Ther. 96: 99 – 113.

[13] C.C. Smith, J. Pharmacol. Exper. Ther. 100 (1950) 408-420.

[14] M.N. Ghosh. Fundamentals of Experimental Pharmacology, 2nd Edition. Scientific Book Agency, Calcutta (1984) 153-8, 187-190.

[15] Olwa-Odyek. Personal communication, Dept. of Pharmacy, Makerere University (1999).

[16] K.M. Loban and E.S. Polozok. Malaria, Mir Publishers, Moscow (1985) 207-243,

[17] G.J. Amabeoku and O. Chikuni. Eur. Neuropsychopharmacol. Mar, 3 (1993) 37 - 44.

[18] W.D. Reid. Pharmacology and the Future of Man, Proc. 5th Int. Cong. Pharmacology, San Francisco (1972) pp. 62-74.

[19] N.K. Mubiru, A.M. Alia, C.A. Amai, S. Apio, G. Basalidde, A.B. Kakooko, J.B. Magimbi, J.B. Mutyaba, J.Z. Nugga, and J.W. Ogwal-Okeng. Ethnobotanical and Traditional Healers Survey, Ethnomedicine in Uganda Series, (1994) Part 1-25.