The determination of safety of Muhanse M4® , a traditional herbal preparation used to treat HIV/AIDS-related conditions and diseases in Tanzania

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Abstract: Muhanse M4® is a traditional herbal preparation that has been in use in Tanzania for the past 17 years to improve the quality of life among people living with HIV/AIDS. This study was carried out to determine the safety of the extract Muhanse M4® in animal models. The qualitative test to identify alkaloids and saponins compounds was carried out. The toxicity tests in Swiss albino mice and rats were done according to WHO guidelines of 1993. Muhanse M4® was dissolved homogeneously in distilled water and was administered both intraperitonially and orally for 14 days for sub-acute test and 24 hours for acute test. Qualitatively, the extract was found to contain no alkaloids or saponins. In rats intraperitoneal doses that caused 100% lethality were 758.55 mg/kg and 553.7415mg/kg when administered singly and repeated, respectively. Single oral dose up to 3034.200mg/kg did not cause any death in the tested mice or rats. NOEL during intraperitoneal repeated doses for liver in rats was 424.788mg/kg, and NOAEL was 455.130mg/kg. In rats $LD_{10\%}$, $LD_{50\%}$ and $LD_{100\%}$ were 485.472mg/kg, 526.4337mg/kg and 553.7415mg/kg, respectively. In conclusion, Muhanse M4® extract is considered to be safe in laboratory animals.

Key words: Muhanse M4®, toxicity, medicinal plants, traditional medicine, HIV/AIDS

Introduction

Human Immunodeficiency Virus/Aquired Immunodeficiency Syndrome (HIV/AIDS) is still one of the major public health problems particularly in the sub-Saharan Africa. In Tanzania about 63,000 AIDS cases were estimated to have occurred in 2002 alone and a cumulative total of 786,000 AIDS cases since 1983 (NACP, 2002). For many years, people living with HIV/AIDS (PLWHA) have had no proper drug for the management of the disease until 2004 when the government started providing Anti-retroviral drugs to about 44,000 AIDS cases. However, a variety of medicinal plants have been used for the treatment of HIV/AIDS in the country.

Muhanse M4® is a traditional herbal remedy made from a mixture of aerial, stem backs and root backs of 10 plants. It is believed that the medicines, was used by local ancestors to treat "Lugandaganda" disease, which presented with similar symptoms and signs like HIV/AIDS particularly, against severe weight loss, patches of skin discoloration and severe dehydration leading to scaling of the skin (L. Mwinuka, *per comm*). Many people in Dar es Salaam, Tanzania have been using Muhanse M4® for treatment of HIV/AIDS related diseases for over a decade now. However, there has been no study to ascertain its safety. Due to this reason this study was carried out to determine the safety of the concotion in animal models.

Materials and Methods

This study was carried out from June-December 2004. It comprised of preparation of water and ethanol extraction of Muhanse M4® followed with intensive toxicity testing in albino mice and rats. A 500g of Muhanse M4® powder (Courtesy: Munufu Traditional Medicine and Research Clinic) was soaked at room temperature in distilled water for 48 hours. The aqueous extract was freeze-dried and 23.599g (4.72%) of dried aqueous extract was obtained. The calculation was done in order to get the approximate dose used by clients. Three tablespoons of powder was weighed and found to have 45g, equivalent to 2.12394g per person per 24 hours; being equivalent to 30.342mg/kg body weight per 24 hours (10.114mg/kg per 8 hours) for a person of 70kg body weight.

The crude extract was evaluated for its toxicity in laboratory animal species including Swiss albino mice and rats according to WHO guidelines (WHO, 1993). The toxicity profile of crude extract was

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determined *in vivo* in mice and rats (WHO, 1993). The extract was dissolved homogeneously in distilled water, the solvent, which does not alter the absorption of test material. A total of 490 mice and 570 rats were used during this study.

During acute phase a total of 130 male and female young adult mice (4-6 weeks of age) of average body weight 20g were randomised into 13 groups, each having 5 males and 5 females. Doses of crude extract ranging from 30.342-606.84mg/kg in distilled water were administred by intraperitoneal injection and 30.342-3034.2mg/kg by oral gavage. Likewise, 5 male and 5 female adult rats (average body weigth= 250mg) were randomised into groups of 10 and the respective doses of crude extracts were administered by intraperitoneal injection and oral gavage. Animals that died during the observation period, as well as those survived to the end of the observation period were autopsied. Brain, lungs, liver, kidney and mesentery specimens were examined for any pathological lesions.

During sub-acute phase animals were being administered with Muhanse M4® extract single dose daily for 14 days. A total of 13 groups of male and female young adult mice (average body weight 20g) were randomized into groups of 5 each (total of 10 per group) and administered through oral gavage doses of crude Muhanse M4® extract ranging from 30.342–1168.167mg/kg dissolved in distilled water. Similarly for intraperitoneal injection doses ranging from 30.342 –447.5445mg/kg were administered to 9 groups of mice.

A total of 15 groups of male and female adult rats of average body weight 250g were randomized into groups of 5 each (total of 10 per group) and doses of crude Muhanse M4® extract in distilled water ranging from 30.342–1957.059mg/kg were administered by oral gavage. During intraperitoneal injection doses ranging from 30.342 - 553.7415mg/kg were administered to 11 groups of rats.

Muhanse M4® was also analyzed for the existence of alkaloids and saponins (known to be toxic) according to Wang *et al.* (1987). In the test for alkaloids 1ml of ethanol extract was dissolved in chloroform to dissolution, and then filtered through a filter paper. Two drops of dragendoff solution was added to the filtrate. The mixture was then observed for any colour change. Another 1ml of ethanol extract was dissolved in normal saline to dissolution then shaken vigorously.

Results

Both extracts were solid and dark brown in colour. Water extract product was homogeneously soluble in water, while ethanol extract was sparingly soluble, but it dissolved when Dimethyl sulphur oxide (DMSO) was applied first. Following the procedure of test for alkaloids, the test solution retained the reddish-brown colour of dragendoff. This implies that the sample did not contain any alkaloid. In the presence of alkaloids, the solution would become brick-red to black in colour. Moreover, the test mixture did not form any stable foam thus indicating the absence of saponins.

The calculated maximum oral dose to be tested for both mice and rats was 3034.200mg/kg, which was 100 times the dose used in humans per day (24hours). The mixture, however could not dissolve because it reached its saturation point. The maximum dissolved tested dose was 2730.78mg/kg body weight, which was 90 times the human dose per day. No mouse or rat died during the testing.

In acute safety testing the maximum dose for mice used for intraperitonial administration was 606.840mg/kg body weight, which was 20 times the dose used in humans per day. This dose caused 100% lethal effect in mice. Intraperitonial injection doses that lead to 10%, 50% and 80% lethal effect in mice were 485.472mg/kg, 530.985mg/kg and 576.498mg/kg, respectively. In rats the maximum dose used for intraperitonially administration was 758.550mg/kg, which was 25 times the dose used in human per day, this dose caused 100% lethal effect. When administered by intraperitonial injection, the doses that lead to 10%, 50% and 80% lethal effect were 591.669 mg/kg, 637.182mg/kg and 713.037mg/kg, respectively (Figure 1).

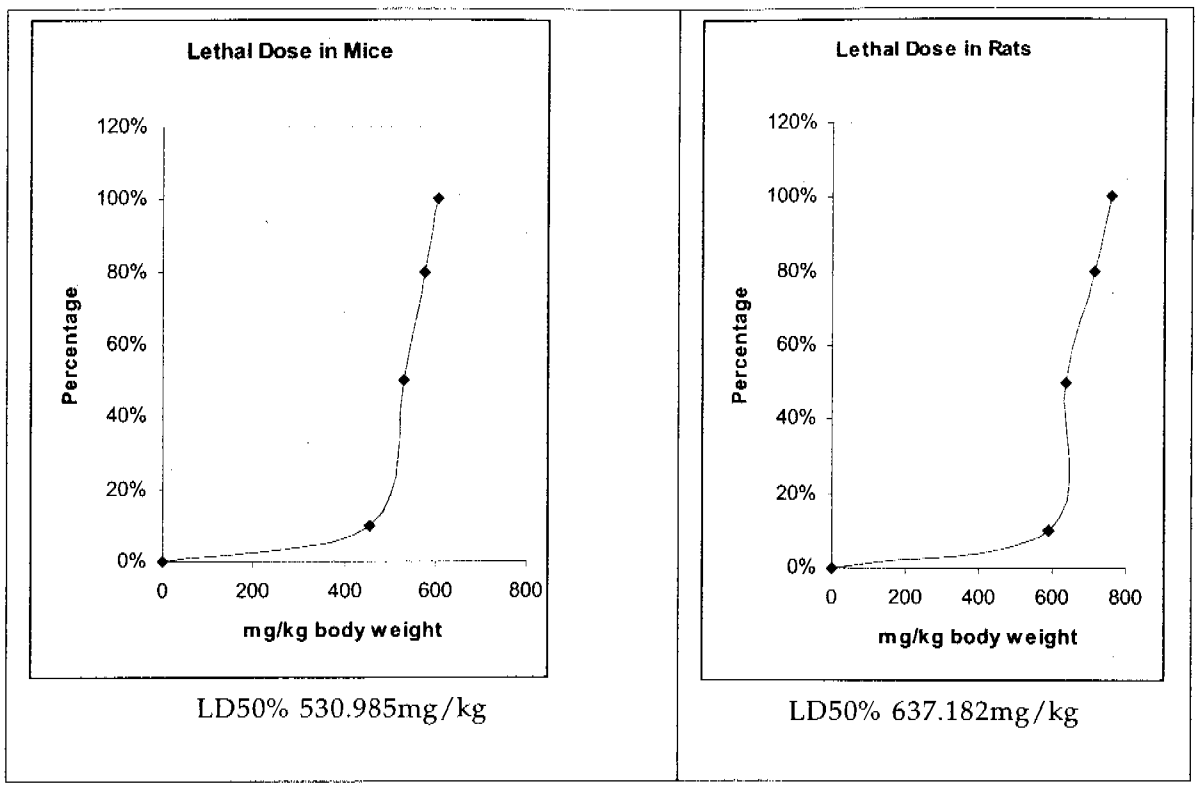


Fig. 1: Acute toxicity of Muhanse in mice and rats when given by intraperitoneal injection

In the mice, the maximum oral repeated dose tested was 1,168.167mg/kg (which is 38.50 times the dose used in humans). Based on NOEL the dose was 819.234mg/kg and NOAEL was 849.576mg/kg, which were 27, and 28 times the human dose. The LD10%, LD50% and LD100% were 910.26mg/kg, 1,092.312mg/kg and 1,168.167mg/kg, respectively.

In rats the maximum repeated doses used was 1957.059mg/kg body weight, which is 64.50 times the human used doses. Based on NOEL the maximum tolerated repeated dose was 1562.613mg/kg and NOAEL was 1608.126mg/kg, which was 51.50 and 53 times the human used dose, respectively. LD10%, LD50% and LD100% were 1668.810mg/kg, 1,835.691 mg/kg and 1,957.059mg/kg, respectively. In mice and rats, the lethal effect was observed on the 3rd and 5th day of administration, respectively (Figure 2a, b).

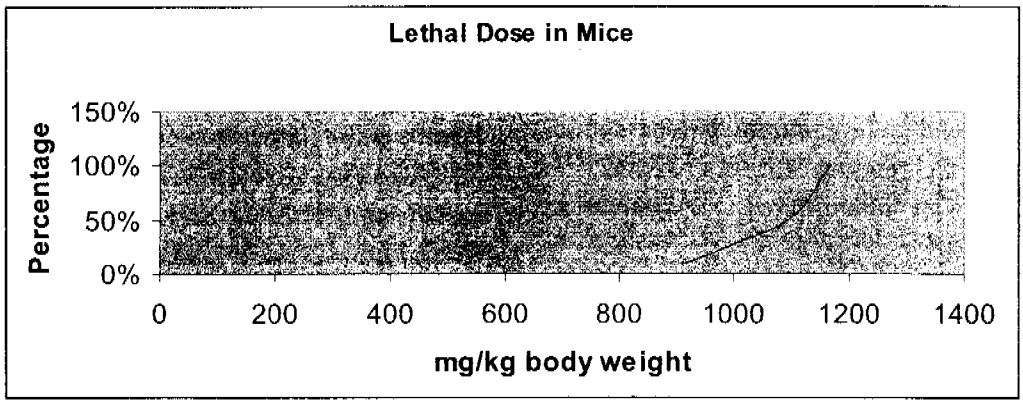


Figure 2a: Sub-acute toxicity in mice when Muhanse was given orally

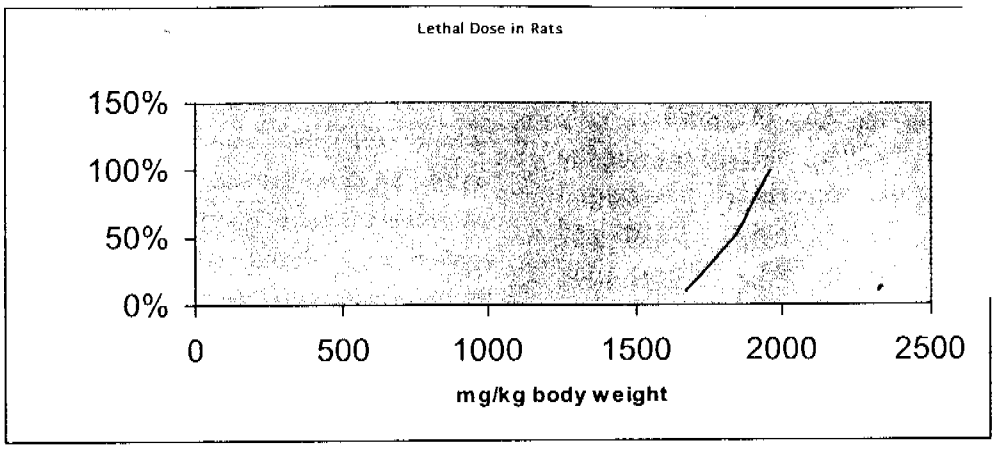


Figure 2b: Sub-acute toxicity in rats when Muhanse was given orally

During sub-acute phase in mice, the oral and intraperitoneal routes exhibited an increase of total

intraperitonially and orally, respectively. Similar changes were observed in rats at doses above

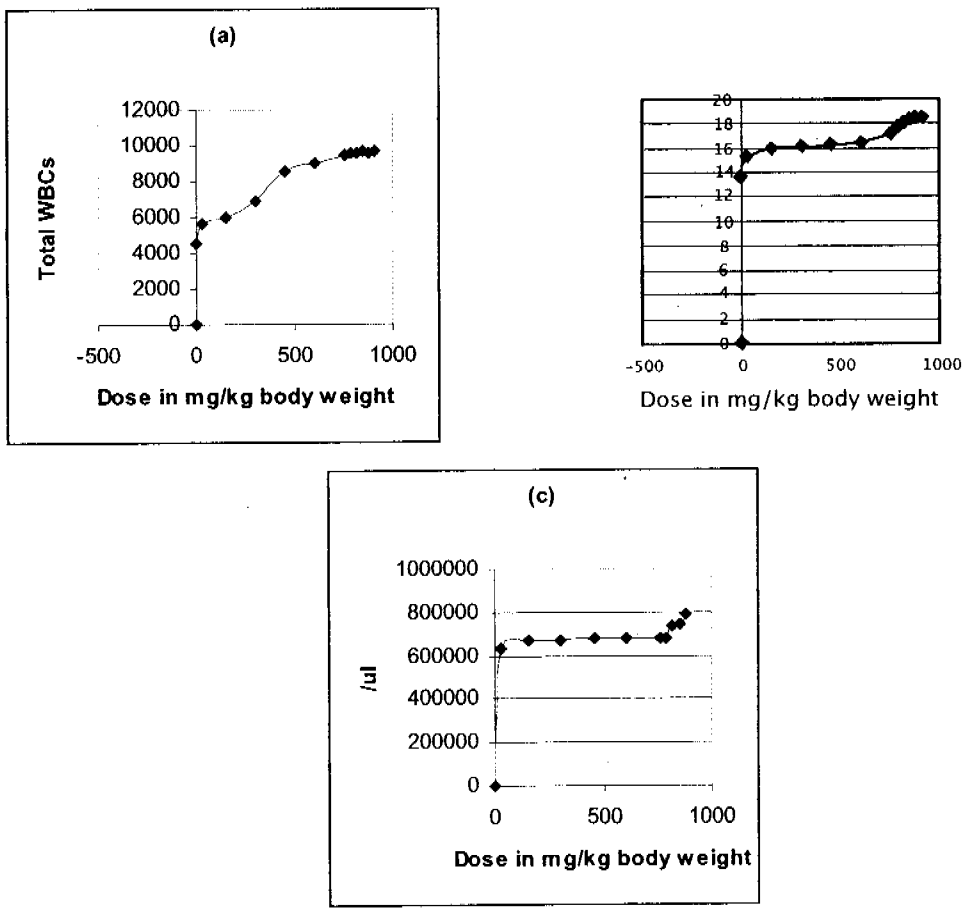


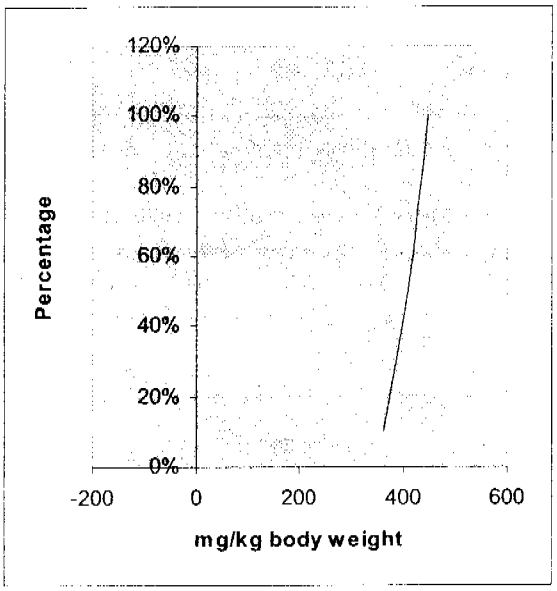
Figure 3: Effect of oral Muhanse M4® on (a) white blood cells; (b) haemglobin; (c) platelets in mice

white blood cells, haemoglobin and platelets levels (Figure 3). However, serum chemistry parameters started to change when the doses were very high beyond NOEL.

Change glutamate pyruvate transaminase and glutamate oxaloacetate transaminase enzymes parameters in mice were observed at doses of above 303.42mg/kg and 819.234mg/kg when administered 424.788mg/kg and 1562.613mg/kg when administered intraperitonially and orally, respectively.

The maximum repeated doses administered intraperitonially in mice was 447.5445mg/kg, which was 14.75 times the human dose. NOEL was 303.420 mg/kg and NOAEL was 333.762 mg/kg, 10, respectively. The LD10%, LD50% and LD100%

were 364.104mg/kg, 409.617mg/kg and 447.5445mg/kg, respectively. In rats the maximum dose administered was 553.7415mg/kg body weight. Based on NOEL and NOAEL, the maximum repeated doses were 424.788mg/kg and 455.130mg/kg, respectively. LD10%, LD50% and LD100% were 485.472mg/kg, 526.4337 mg/kg and



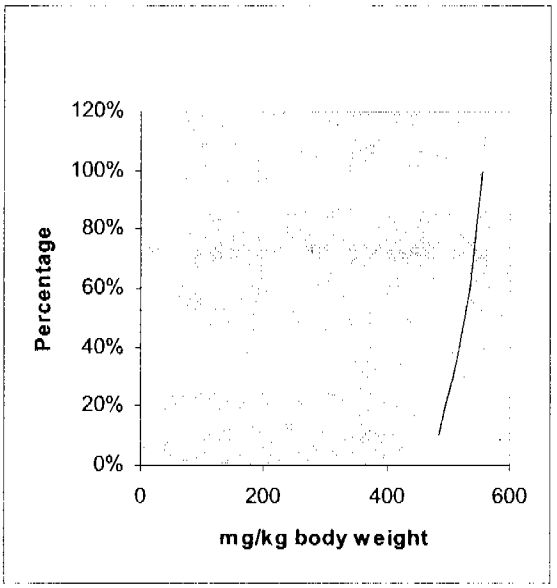


Fig. 4: Sub-acute toxicity in mice and rats when Muhanse was administered by intraperitoneal route

553.7415mg/kg, respectively. In mice and rats, the lethal effect was observed on the 2nd and 4th day of administration, respectively (Figure 4). The safe range in mice and rats during repeated doses administered orally for 14 days singly was 28 and 51.5, respectively. Similarly when administered intraperitoneally the respective safe range were 10 and 14.

During acute phase, thirty minutes post intraperitoneal administration all animals were observed to manifest muscle weakness of the hind limbs characterized by failure to stand, walk or run properly for 1-2 hours. Death of animals occurred in the late hours (between 18-24 hours) post herbal drug administration. Death was preceded by loss of mobility and recumbency and slow breathing rates. At autopsy (after death or at the end of the experiment), examination of the brain, lungs, liver, kidney and mesentery did not reveal any gross pathological changes.

Discussion

Muhanse M4® concoction is taken orally at a dose of one tablespoon eight hourly for a long time depending upon the user. Our findings suggest that the crude extract is non-toxic because water extract applied orally through gavage did not show lethal effect to laboratory animals at a single dose. In the acute phase of the experiment none of the animals died due to the oral use of Muhanse M4® even at a higher dose of 2730.780mg/kg, which is 90 times human dose per day. However, during this acute phase, tested animals exhibited transient muscle weakness of the hind limbs. This is likely to have attributed to drug irritation to the nerve endings in the abdomen probably causing impairment of vegetative nerves to control the innervated muscles. During intraperitoneal acute safety testing, mice and rats were affected at higher doses 16-25 times the human dose per 24 hours. This suggests that the lethal effect was very small.

Our findings indicate that haematological parameters of white blood cells, platelets and haemoglobin increase with an increase in dosage of Muhanse M4®. In addition, liver enzymes glutamate pyruvate transaminase and glutamate oxaloacetate transaminase are highly affected with increase in dosage beyond NOEL. Interestingly, the oral administration of Muhanse M4® extract on mice and rats had no apparent effect on renal

enzymes or haematological parameter. These results suggest that Muhanse M4® extract contains no alkaloids and saponins toxic to human beings. The low toxicity level of Muhanse M4® aqueous extract as exhibited during the study clarifies the traditional use over centuries.

Currently there are no documented records on the use of pure compounds from Tanzanian plants for immune-modulations specifically in HIV victims. However, there is substantial information and anecdotal evidence on the use of crude plant materials for the same. The many plants that are used as immunomodulators in HIV affected individuals are also used to treat other viral diseases such as measles, hepatitis B and influenza. For instance, *Phyllanthus niruri/amarus* and *Cajanus cajan* are currently being used traditionally as remedy for hepatitis B in Tanzania. In India for example *Phyllanthus amarus* is used in the treatment of viral hepatitis and jaundice (Venkateswaran *et al.*, 1987). The active constituents contained in Phyllanthus have been shown to inhibit the surface antigens of some viruses (Venkateswaran *et al.*, 1987).

Our findings, on safety for Muhanse M4® indicate that the extract is tolerable in rodents. However, there is a need to carry further study on two different animal species, one being a rodent (mice, rats or rabbits) and the other one non-rodent (dogs or cats), in order to recommend its use in human for large population.

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