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Short communication

DOCUMENTATION ON THE USES OF *MORINGA STENOPETALA* AND ITS POSSIBLE ANTILEISHMANIAL AND ANTIFERTILITY EFFECTS

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ABSTRACT: Documentation on the uses of *Moringa stenopetala* in Arbaminch and surrounding villages and Wollayeta Sodo area has shown that the fresh leaves are eaten as vegetable. The leaves and the roots are also useful for treating various diseases. According to the practice of the local people, the leaves of *Moringa stenopetala* boiled in water can be used in curing malaria, hypertension and stomach pain. They sometimes use the leaves to expel retained placenta in pregnant women. The roots chopped and mixed with water are used mainly for malaria treatment. In the present study crude ethanol extracts of leaves and roots were tested separately on *L. donovani* promastigotes. The serially diluted crude extracts resulted in abnormal morphologies of the promastigotes after 48 hr of incubation. The dilutions used were high to warrant potent antileishmanial activity of the crude plant extract. The antifertility effect of the leaf ethanol extract in Swiss albino mice was found to be 73.3% taking mating to be 100%.

Key words/phrases: Antifertility, antileishmania, medicinal plants, *Moringa stenopetala*

INTRODUCTION

Moringa stenopetala Lam. is a deciduous plant widely distributed in the southern part of Ethiopia at an altitude range of about 1100 to 1600 meters. The major growing regions are Arbaminch and surrounding areas, Negelle and Wollayeta Sodo area which are about 400 to 550 kms south of Addis Ababa. *M. stenopetala* is commonly called *shiferaw* in Amharic and *aleko* in Wolla-

yetegna and Gamugna. The antimicrobial property of *M. stenopetala* and a related species *M. oleifera*, largely distributed in the Asian sub-continent, has been documented (Eilert *et al.*, 1980). The seeds of *M. stenopetala* have been shown to serve as purifiers of muddy and turbid water in some parts of Africa (Jahn, 1979; Gecaga, 1980; Jahn, 1981) and in Ethiopia (Gottsch, 1984; Aschalew Hundie and Adinew Abebe, 1991). The non-toxic effect of the seeds of *M. stenopetala* in rats has been reported by Berger and co-workers (Berger *et al.*, 1984). This suggests the safe use of the seeds to clear water for domestic consumption.

In this paper, the result of a field survey on the uses of *M. stenopetala* is presented. We also report the observation made on the antileishmanial effects of the ethanol extracts of the leaf and the root on promastigotes of *L. donovani*. The *in vivo* antifertility effect of the ethanol extract of the fresh leaf tested in mice is also reported.

MATERIALS AND METHODS

Documentation on the uses of Moringa stenopetala

Home to home visits were made to enquire about the uses of the plant. The areas covered were small villages and localities in and around Arbaminch, Wollayeta Sodo and Negelle areas. The visits were made in November 1995 and in March 1996. Standard questionnaires were used to collect the data.

Extract preparation

M. stenopetala fresh leaves and wet roots were collected from Arbaminch town and surrounding villages. Representative samples are kept in the National Herbarium of the Addis Ababa University. The fresh leaves and wet roots were ground into small pieces separately. In 1:9 (w/v) proportions the ground pieces were macerated in 90% ethanol and kept in stoppered flasks by continuous shaking for 48 hr. After filtration the alcohol was allowed to evaporate using a rotavapor and the semi-solid precipitate was then taken as the ethanol extract and kept at -20° C until used.

Leishmania culture

L. donovani MHOM\ET\89\IPB 399 promastigotes (obtained from the Institute of Pathobiology, Addis Ababa University) were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum with 5 $\mu l(ml)^{-1}$ of a mixture of 1% penicillin and streptomycin at about 25° C. About $1x10^6$ promastigotes per well, counted with haemacytometer, were transferred to 24-well tissue culture plates, Falcon 3047. Serial dilutions of 6000, 3000, 1500, 750, 375, 187.5 $\mu g(ml)^{-1}$ were prepared in RPMI medium from a stock solution of 60 $mg(ml)^{-1}$ of the leaf or the root extracts separately. Each dilution was tested in triplicate in the wells containing the promastigotes. Four wells seeded with the promastigotes were randomly selected from each plate and used as controls without the addition of extracts. After 48 hr of incubation the motile promastigotes were counted in each well and any change in the morphology was observed. Change in morphology was considered to be any alteration in appearance observed in comparison to the motile promastigote form. Loss of the flagella and a roughly circular to oval shape were considered to be the major abnormal morphologies. Statistical analysis of the serial dilutions was performed using Chi square test of proportions at an alpha value of 0.05 (Fleiss, 1973).

Antifertility test

Swiss albino mice bred in the animal house of the Department of Biology were used. Four weeks old new-born mice separated from their mothers were fed *ad libitum* until they were two months old. Experimental and control mice were kept in separate cages following Williamson *et al.* (1996). The experimental female mice were given the extract in doses of 200 mg/kg body weight orally while equivalent solvent vehicle was given to each control mouse. The extract was dissolved in 0.5 ml ethanol/distilled water mixture (0.2:0.3). Male mice were introduced into each cage after 8 days of dosing the females with the extract. The extracts were continually given to the females. The males were removed from each cage after 8 days. The extract application to the females continued until the 19th day, counting the days from the day the males were introduced into the female cages. To ascertain that each mouse did mate with the male body weights were recorded every day starting the first day of the experiment. All female mice both in the test and the control groups were sacrificed on the 20th day. The number of implantation sites and fetuses were recorded.

RESULTS

Fifty one house holds were visited during the documentation period. The visits in the different localities have shown that almost every household has at least one or two *Moringa* plant in its compound. Enquiries were made on how the people use the different plant parts. As is summarized in Table 1, the leaves, the roots and the seeds have at least one or more uses for the community. Our documentation on the use of the plant based on the responses obtained has shown that, the fresh leaves of the plant cooked and seasoned with oil and salt are eaten as vegetable. The local people also claim that the leaves boiled as tea or chopped and mixed with water are used in the treatment of malaria, hypertension, stomach problems, expulsion of retained placenta and in some other health problems like asthma and diabetes. The wet or the dried root part chopped and mixed with water is also used to treat malaria. The seeds are used in clearing muddy water especially in a village called Kola Shara near Arbaminch. A handful of seeds are taken and put in a bucket containing about 20 litres of muddy water. After about an hour all the dirt accumulates at the base of the bucket and settles down. The seeds serve as adherents to coagulate all the impurities in the turbid water. We also observed ladies selling fresh cut leaves of the plant in the markets.

Table 1. List of the different uses of *Moringa stenopetala* expressed as the number of responses obtained from enquiries of individuals from 51 households.

Uses	Leaves	Root	Seeds
Food	30	-	-
Malaria	16	21	-
Hypertension	17	-	-
Stomach problem	10	1	-
Retained placenta	8	-	-
Clearing water	-	-	4
Asthma	3	-	-
Diabetes	2	3	-
Common cold	2	-	-
Wound healing	1	-	-

The dose-dependent antileishmanial effects of the leaf and the root extracts on *L. donovani* promastigotes are shown in Table 2. At higher concentrations ranging between 6000 and 1500 $\mu g(ml)^{-1}$ no motile promastigotes were observed. The percent abnormal morphologies (after allowing for those with normal morphology) obviously decreased with the decrease in concentration of the extracts. The root ethanol extract exhibited higher abnormal morphology percent for every serial dilution as compared to the leaf ethanol extract. However, the difference is not remarkable to suggest the high potency of the root ethanol extract. The control promastigotes were highly motile and maintained their normal morphology throughout the incubation time of 48 hr in both the leaf and the root extracts.

Table 2. Effect of *M. stenopetala* leaf and ethanol extracts given as the percent abnormal morphology with the 95% confidence intervals shown in parentheses. Values are means of three independent experiments.

Conc. [$\mu g(ml)^{-1}$]	Abnormal morphology (%)	
	Leaf	Root
6000	85.03 (0.06)	94.18 (0.04)
3000	66.67 (0.09)	68.64 (0.08)
1500	42.65 (0.09)	51.13 (0.05)
750	18.64 (0.07)	26.84 (0.08)
37 5	9.32 (0.05)	17.80 (0.07)
187.5	5.36 (0.04)	8.19 (0.05)

The antifertility effect of the fresh leaf ethanol extract in the Swiss albino mice is given using the following relationship:

$$\frac{\textit{No. of implants in control} - \textit{No. of implants in test group}}{\textit{No. of implants in control group}} \times 100$$

Based on the above relationship the antifertility effect of the leaf ethanol extract was 73.3% (Table 3). This takes into consideration that each of the test mice had mated with a male.

Table 3. The percent antifertility effect of *M. stenopetala* leaves in Swiss albino mice. The number of mice in each group was 5.

Expt	Number of implants		Antifertility (%)
	Control	Test	
1	5/5	3/5	40
2	3/5	0/5	100
3	5/5	1/5	80
Average	13/15	4/15	73.33

DISCUSSION

The fact that the *Moringa* plant is widely used as a herbal medicine in areas where visceral leishmaniasis prevails (e.g., Konso, southern Ethiopia) (Teklemariam Ayele and Ahmed Ali, 1984; Asrat Hailu and Frommel, 1993) was a good ground to study the possible antileishmanial property of its leaf and root. The ethanol extracts of both the leaves and the roots showed a change in the morphology of the *L. donovani* promastigotes which was either loss of the flagella or a roughly circular to oval appearance of the promastigotes as compared to the respective controls. Other plant species were reported to exhibit a change in the morphology and to arrest flagellar motility of *L. donovani* promastigotes. A good example is the alcohol extract of the Chinese licorice roots which inhibited the growth of *L. major* and *L. donovani* promastigotes (Chen *et al.*, 1993). Iwu *et al.* (1992) have evaluated the antileishmanial potential of extracts of eleven plants and reported the positive finding of five plants. Still other recent workers substantiate the importance of plants as sources of antileishmanial drugs (Fournet *et al.*, 1994; Sahpaz *et al.*, 1994). It is evident from Table 2 that the serial dilutions used were apparently high. However, the extracts dose dependently altered the normal parasite morphology. The ethanol extracts were not effective at the lower concentrations as reported from high efficacy antileishmanial plant extracts (Azeb Tadesse *et al.*, 1993). Nevertheless, the *M. stenopetala* leaves and roots may be exploited with further isolation and characterization of the active component(s). It is possible that the

isolated active ingredient may serve to potentiate the efficacy of other anitleihamanial drugs of plant origin.

The antifertility effect of the leaf ethanol extracts is observed through the percent reduction in the implantation sites of the mice. In another set of experiments isolated uteri from mice and guinea pigs tested in organ bath containing the leaf ethanol extracts showed enhanced contractions (data not shown). This indicates the possible facilitation of increased uterine smooth muscle contractions by the extract, which eventually may result in rejection of implantations. Thus, the use of Moringa leaves to expel retained placenta by women may be related to the increased uterine smooth muscle contractions induced by components of the leaves.

The present finding could be compared to the related species of *M. oleifera*, where the dried leaf ethanol extract induced abortion in female albino rats (Nath *et al.*, 1992). The abortive property of *M. oleifera* has been synergized in combination with *Adhatoda vasica*. This gives a good clue or possibility for the potentiation effect of the *Moringa* species to serve as an antifertility and/or abortifacient plant in combination with other plants. The antifertility property of the aqueous extract of the roots of *M. oleifera* in rats was reported by many investigators (Parakash *et al.*, 1987; Shukla *et al.*, 1988; Shukla *et al.*, 1989). It can not be claimed that both *Moringa* species may have equally potent antifertility components, but the possibility that both species may share some similar components can not be overruled. However, this requires further verification.

In conclusion, the documentation on the uses of Moringa is a useful information on its biomedical diversity. Moreover its diverse use has to be studied further and justified. This preliminary experimental result could be substantiated with a further thorough study of the other plant parts using various extraction and isolation methods. The final isolation and characterization of the active compound(s) would then be crucial to consider the plant as medicinally useful.

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