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Full Length Research Paper

Efficiency of ethanol extract of *Moringa oleifera* lam leaves for the treatment of *Staphylococcus aureus* infections in chicks

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The efficiency of ethanol's extract of *Moringa oleifera* was evaluated in *Staphylococcus aureus* infection on Harco chicks. For this, 150 Harco chicks divided in 5 lots were used: A negative control (not inoculated and not treated); 0.5 g/L; 1.5 g/L of *M. oleifera*; 0.5 g/L of Flumequine®50 and a positive control (inoculated but not treated). The test of χ^2 was used for the comparison and the test of student for the means comparison between lots. *In vitro* 1.5 g/L of the extract shows a positive result on *S. aureus*. However only 0.5 g/L of the extract has proved an efficiency *in vivo*. Compared to the other treatments, only 0.5 g/L of *M. oleifera* has allowed a daily gain of weight during the 4th period (11.4 +/- 1.68). There was a significance difference at 1% for food consumption. The treatment of 0.5 g/L of *M. oleifera* has decreased the leukocytes number from 5363333.33 to 4383333.33/ ml of blood.

Key words: Efficiency, *Moringa oleifera*, *Staphylococcus aureus*, Harco chicks.

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic micro-organisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries (Al-Bari et al., 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies in animals' health field (Zy et al., 2005; Rojas et al., 2006). *Moringa oleifera Lam.* is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005) which is widely used for treating bacterial infection, fungal infection, anti-infammation, sexually-transmitted diseases, malnutrition and diarrhea in human and animals. The aim of this study is to evaluate the efficiency of *Moringa oleifera*'s ethanol extract in *Staphylococcus aureus* infection on Harco chicks.

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MATERIALS AND METHODS

Area of study

The extraction of *M. oleifera* and the tests *in vivo* were respectively realised in the laboratory of pharmacognosy and essentials oils and at experimental farm of Animal Health and production at the University of Abomey – Calavi in Benin. The tests *in vitro* were performed at the National Laboratory for Public Health at the Ministry of Health of Benin from October 1st to December 31st,

2010. The vegetal material constituted of leaves of *M. oleifera* authenticate at the National Herbier of Benin as AA6282/UNB. Flumequine®50, an anti staphylococcus for poultry often used by farmer was used as control. The biological material was a strain of *S. aureus* ATCC®25923. A total of 150 of Harco chicks which are one week ago. They were provided by the hatchery of "Poussins du Roi" at Pahou. There was no *S. aureus* infection 6 months before the tests in that hatchery.

Ethanol extraction of *Moringa oleifera*

One hundred grams of fresh leaves of *M. oleifera Lam.* were weighed out and crushed directly by grinder and dipped into 400 ml of ethanol into a conical fask stoppered with rubber corks and left for 7 days with occasional shaking. The standard extracts obtained were then stored in a refrigerator at 4°C for antibacte rial activity test (Akueshi et al., 2002).

Antimicrobial tests

For the tests *in vitro*, a range of dilutions from 10^{-1} to 10^{-6} of bacterial suspensions in sterile nutrient broth was prepared. On each dilution, a variable quantity of the extract of *M. oleifera* was added. After 24 h of incubation at 37°C, the dilutio ns were inoculated on agar plates of Muller Hinton for bacterial counts. The numbers of surviving bacteria obtained from different dilutions containing the extract of *M. oleifera* were compared to the number of bacteria obtained with the dilution without the extract in order to determine an eventual microbial effect.

A number of 10 chickens were used for the inoculation. Each chick received a minimum infective dose of 80.10^5 bacteria / ml by intramuscular. We obtained 8 chickens dead; so we adopted a dose of 66.10^5 bacteria / ml by intramuscular to inoculate each chicken of groups 2, 3, 4 and 5.

Experiments' animals

The *in vivo* tests were performed on the chicks (150). The first group was constituted of chicks which were neither inoculated nor treated; the second was the chicks which received 0.5 g/L of the extract of *Moringa oleifera*; the third group was treated with 1.5 g/L of the extract of *Moringa oleifera*; the fourth was treated with 0.5 g/L of Flumequine®50 and the fifth group was inoculated but not treated. All of those substances were given to the chicks by mixing them with water.

A haematometer was used to count the leukocytes for each group of chicks before the inoculation, 3 days after the inoculation and 7 days after treatment.

Zoo technical parameters

The quantity of food consumed feed intake, the weight of each week, the average daily gain (ADG), the feed efficiency feeding index and the mortality rate were evaluated.

Statistical analysis

The blood and the zootechnical parameters were recorded. Software SAS (Statistical Analysis System, 1996) was used for the data analysis. The variance analysis was carried the lot of chicks as source of variation. The test of χ^2 was used for the comparison of mortality rate between lots and the test t of student for the means comparison between lots.

RESULTS AND DISCUSSION

According to the results, we have a few growth of bacteria with the concentration of 0.5 g/L of water of *M. oleifera* and very few growth with 0.5 g/L of (Flumequine®50) and 1.5 g/L of *M. oleifera* (Table 1). About the feed consumption, there is difference between the lots at the end of experiences (Table 2). There is significant difference at 1% ($p>0.001$) between the lots about weight (g) and average daily gain (g/j (Table 3) but no difference about the feed efficiency (Table 4). There is more mortality of chicks with the treatment of 1.5 g/L of *M. oleifera* (Table 5) but no difference about leucocytes number between the lots (Table 6).

In vitro and *in vivo* test

The present study was conducted to obtain preliminary information on the antibacterial activity of ethanol extracts of *M. oleifera Lam*. leaves in Benin. This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair et al. (2005). The number of bacterial colonies has progressively decreased when *M. oleifera* extract concentration has increased. Therefore, the colonies are rare at 1.5 g/L of the extract (Table 1). This observation is similar to the results of Georges and Pandalai (1949). They noticed that *M. oleifera* is efficient on *Escherichia coli, S. aureus* and *Salmonella spp.* By contrast, this result is different with the results of Marquis et al. (1977) who had shown that *M. oleifera* leaves could not inhibit *S. aureus* development.

The difference between these results could be explained by the solvent. In this study, ethanol was the solvent instead of water used by Marquis et al. (1977). There was no significant difference for the number of leukocytes. Nevertheless the number of leukocytes for the second batch has dropped from 5363333.33 to 4383333.33 / ml after treatment. This diminution could be caused by the extract as it could have reinforced the chicks' immunity. This variation has confirmed the results of Calleja (1998) who affirmed that if there is diminution of the leukocytes number after an antibiotic treatment, it could have a beneficial effect as of the interference on the immunity system (Table 6). These consequences suggest that *M. oleifera Lam.* leaves used contain bio-components whose antibacterial potentials are against Gram-positive bacteria tested. The activity of the plant against Gram-positive bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007). Moringa leaves have been reported to be a good source of natural antioxidants such as

Table 1. Efficiency of ethanol extract of *Moringa oleifera* on *Staphylococcus aureus.*

Extract concentration	Colonies on agar Mueller Hinton
0.5 g/L (*Moringa oleifera)*	++
0.5 g/L (Flumequine®50)	+
1.5 g/L (*Moringa oleifera)*	+

++: Few growth of bacteria +: Very few growth of bacteria.

Table 2. Distribution of the different lots according to the quantity (g) of food consumed in time.

Lots					Level of signification
A	B	C	D	E	
74.24^{a}	74.23^{a}	74.24^{a}	74.24^{a}	74.24^{a}	*
140.00^{a}	139.90^{a}	139.90^{a}	139.70^{a}	140.00^{a}	*
147.57^{a}	148.63^{a}	148.63^{a}	148.63^{a}	147.57^{a}	*
159.91^{a}	162.14^{a}	163.1^{a}	162.09^{a}	159.59^{a}	*
136.17^{a}	142.14^{a}	142.14^{a}	141.69^{a}	141.61^{a}	*
261.40^{a}	260.06^{a}	262.06^{a}	265.22^{a}	261.08^{a}	*
292.06^{b}	277.63^{a}	277.63^{a}	277.63^{a}	277.63^{a}	*
298.50^{a}	294.22^{a}	295.22^{a}	296.68^{a}	295.22^{a}	*
270.67^{a}	247.96^{b}	247.96^{b}	251.58^{b}	237.50^{a}	**
197.83±0.27^{a}	193.55±^{b}	194.55±3.76^{bc}	195.27±1.81^{b}	192.71±0.83^{b}	**

**: Significant difference at 1% (p<0.001) *: Non significant difference at 5% (p>0.05).

Table 3. Weight (g) and average daily gain (g/j) of the chicks according to the lot.

Variable	Lots					Signification test
	A	B	C	D	E	
P_0	36.00^{a}	38.00^{a}	35.30^{a}	33.30^{a}	30.00^{a}	*
P_1	70.41^{a}	69.00^{a}	70.41^{a}	65.00^{b}	65.33^{a}	**
P_3	153.00^{a}	150.00^{a}	152.26^{a}	153.91^{a}	138.54^{b}	**
P_6	278.12^{a}	353.12^{b}	317.19^{a}	312.49^{a}	362.50^{b}	**
P_9	544.16^{a}	591.66^{a}	481.33^{b}	540.00^{ab}	552.75^{b}	**
GMQ_{10}	4.92^{a}	4.43^{a}	5.01^{b}	4.53^{a}	5.05^{b}	**
GMQ_{11}	5.90^{a}	5.86^{a}	5.85^{a}	6.35^{b}	5.23^{a}	**
GMQ_{13}	5.96^{a}	9.67^{b}	7.85^{a}	7.55^{a}	10.66^{b}	**
GMQ_{16}	12.66^{b}	11.36^{b}	7.82^{a}	10.83^{b}	9.06^{b}	**
GMQ	12.67±0.40^{a}	11.36±1.68^{a}	7.82±1.68^{b}	10.83±1.68^{b}	9.06±1.68^{b}	**

W_i: week I, **: Significant difference at 1% (p<0.001) *: Non significant difference at 5% (p>0.05).

ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German, 2000). These components are useful for animals' immunity.

Zoo technical parameters

The quantity of food consumed per chicks during the first week of the experimentation was comparable to the amount advocated by Blum (1989) who suggested that a chick should have eaten 1820 g of food by the end of the eighth week. The fact that the chicks from the second batch had the same feeding behaviour as the control could be explained by the treatment they received which suggested a beneficial effect from the extract resulting in the reduction of the number of *S. aureus* for 0.5 g/L dose. This beneficial effect had been proved by Sofowora (1996).

There was a significant difference for the weight at the first and the nineth week (P<0.05). The difference of the

Table 4. Feed efficiency.

Period	Lots				
	A	B	C	D	E
1st W	2.16[a]	2.39[a]	2.11[a]	2.34[a]	2.1[a]
2 et 3RD W	3.48[a]	3.49[a]	3.52[a]	3.24[a]	3.92[a]
4, 5 et 6TH W	4.45[a]	2.77[b]	3.44[b]	3.59[b]	2.51[b]
7, 8 et 9TH W	3.23[a]	3.59[a]	5.0[b]	3.63[b]	4.25[b]
Average	3.33±0.94[a]	3.06±0.58[a]	3.52±1.18[a]	3.20±0.60[a]	3.20±1.05[a]

The means of the same line followed by different letters differ significantly at 5%.

Table 5. Mortality of chicks.

Lot	Number	Mortality	Mortality rate (%)
A	30	0	0[b]
B	30	0	0[b]
C	30	4	13.33[a]
D	30	0	0[b]
E	30	0	0[b]

The mortality rates of the same column followed by different letters differ significantly at 5%.

Table 6. Mean of leukocytes per chick and per lot (10^5).

Variable	Lots					DSR	Signification test
	A	B	C	D	E		
Before inoculation	26.8[a]	31.3[a]	29.5[a]	25.6[a]	23.0[a]	32.7	NS
During: three days after inoculation	56.2[a]	53.6[a]	62.9[a]	53.0[a]	49.0[a]	90.0	NS
After the treatment	44.8[a]	43.8[a]	51.2[a]	47.9[a]	53.1[a]	80.2[a]	NS

NS: Non significant difference at 5% (p>0.05), Before: before inoculation, During: three days after inoculation and After: after the treatment.

first week was due to the non valorisation of food by the fourth and the fifth group of chicks (Table 3). The chicks of the second group have valorised their food resulting on the lowest feeding index of 3.06. This index is higher than the results of Petit (1991) on 9 weeks old chick. This difference could be explained either by the stress caused by the experimentation on the chick especially the sampling of blood to count leukocytes or by the inoculation of the bacteria as antigen (Table 4).

The mortality of chick during the experimentation (9th week) are resumed in Table 5. The mortality of the third batch is the highest of 13.33%. This high mortality could be explained by the high concentration of the extract of 1.5 g/L. According to the results of Gupta et al. (1989) and confirmed by our observations, the leaves of *M. oleifera* contained tannin which is an anti feeding chemical.

Conclusion

The ethanol extract of *M. oleifera* has proved an antibacterial effect *in vitro* and *in vivo* on *S. aureus.* The extract was as efficient as Flumequine® 50 at the same dose. Thus, *M. oleifera Lam.* could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

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