Effects of Paronychia kurdica on teat and udder papillomatosis in cows

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SUMMARY

The efficiency of Paronychia kurdica extract on teat and udder papillomatosis was investigated in 30 cows and heifers, which were randomly allotted in 5 equal groups. The plant extract was administered to animals orally at 2 dosages (5g and 10g, groups 1 and 2), subcutaneously (5g, group 3) or percutaneously as a pomade including 10% of Paronychia kurdica (group 4) whereas cows from the 5th group were untreated and served as negative controls. After a 90 day long experimental period, the proportions of completely cured animals (50% and 83.3%) were significantly more elevated when cows were orally or subcutaneously treated than in controls or in animals completely cured animals (50% and 83.3%) were significantly more elevated when cows were orally or subcutaneously treated than in controls or in animals treated with the pomade. Furthermore, wart disappearance occurred earlier when cows were orally or subcutaneously treated than in controls or in animals. After a 90 day long experimental period, the proportions of completely cured animals were significantly more elevated when cows were orally or subcutaneously treated than in controls or in animals treated with the pomade. Furthermore, wart disappearance occurred earlier in these 3 groups (on days 5 and 10 vs. day 15 in groups 4 and 5) and was significantly intensified since the 15th day. In addition, wart losses were maximal in cows receiving oral treatment with 10g Paronychia kurdica extract. In parallel, high contents of unsaturated fatty acids (oleic and linoleic acids) and anti-oxidants (α-tocopherol, linol and linalool) were identified in the plant extract throughout gas chromatography and mass spectrometry. These results demonstrated beneficial effects of the Paronychia kurdica extract administered orally or subcutaneously on udder papillomatosis in cows but the identification of active compounds need further investigations.

Keywords: Papillomatosis, Paronychia kurdica, cow, udder, wart, remission.

Introduction

Papilloma viruses cause epithelial tumours in many animals and humans. Bovine papillomavirus (BPV) induces papillomas of cutaneous or mucosal epithelia in cattle [6, 21]. The papillomas are benign tumours and generally regress but occasionally persist and provide the base for malignant transformation into squamous-cell carcinoma, particularly in the presence of predisposing environmental cofactors [21]. Six serotypes of the virus have been reported to produce bovine papillomatosis [7, 21] and among them, the papilloma types 5 and 6 viruses can cause bovine teat papillomatosis [21]. Generally, the epithelial defects caused by the virus are not so important unless the warts become so large that milking becomes difficult [22]. However, fibropapillomas appear in the duct capillaries because BPV may result in the suppression of natural defence mechanisms of the udder and, therefore, they may be a predisposing factor for mastitis. In addition, animals with warts may be disqualified from shows and exhibitions [7, 22].

Papillomatosis usually appears on the body surface of cattle over 2 years of age. However warts can occasionally be found on the teats of lactating cows. Calves are readily infected by the papilloma virus entering through the broken skin. Warts will appear within one to six months after virus inoculation [2]. Carrier cattle are the main source and natural reservoir of the virus but halters, ropes and instruments can serve as potential sources of infection as well [7, 17]. Infection by BPV results in the onset of benign proliferative lesions in cattle that usually regress spontaneously by a cell mediated immune response. Occasionally, warts persist as benign tumours and progress to squamous cell carcinoma or urinary bladder transitional cell papilloma [18, 19].

Vaccines that prevent or cure BPV infection can provide a potential model for formulating vaccines against human papillomaviruses.
papilloma virus [7]. Warts usually shrink and drop off after a few months of appearance. The spontaneous recovery has probably been the basis for the alleged effectiveness of many treatments including application of several kinds of oil, organic acids, wart pinching or surgery. Any of these appear to be effective if the warts regress spontaneously [5]. Alternative suggested treatments for removing warts use keratolytic (desquamating) agents or local antiviral drugs such as acyclovir, ribavirin and cidovifir [11]. One of the traditional methods for treatment of teat papillomatosis in some rural areas of Turkey involves using wart weed (Paronychia kurdica) plant. As a local treatment method, wart weed has some therapeutic effects, including hypoglycaemic and diuretic actions [1, 3, 14, 20], treatment of kidney stones [9], and cancer suppressive effects [12, 13]. In the present study, the therapeutic effects of applying wart weed (Paronychia kurdica) in treating bovine teat papillomatosis are investigated.

Materials and Methods

PLANT MATERIAL AND EXTRACT PREPARATION

The wart weed plant (Paronychia kurdica) was collected from the provinces of Bingol and Elazig located in Eastern Anatolia in early summer of 2005. The plant was identified and authenticated using descriptive literature and comparison with the herbarium collection in the Department of Biological Sciences at Faculty of Sciences of the University of Firat.

The extract was prepared by mixing 5.10 g of the coarsely powered aerial parts of Paronychia kurdica with 100 mL tap water for 20 minutes and filtering twice through No. 4 filter paper at room temperature. The freshly prepared extracts were used in the experiments immediately after cooling. The use of distilled water for the extraction was omitted, since the herbalists recommend the use of these traditional medicines after boiling in tap water.

ANIMALS AND PROTOCOL DESIGN

Thirty cows (average age: 2.25 years) suffering from teat papillomatosis from various cattle herds were included in the present study. First, warts were observed on the teats of cows. Then, their size, shapes, location and numbers were precisely recorded and clinically examined in order to ensure that the animals were actually infected with papillomavirus. For this purpose, morphology and histopathology of the warts were examined with minimal manipulation. Thereafter, the animals were randomly assigned to 5 equal groups (6 cows in each group) according to the dosage and the route of administration of the plant extract; whereas cattle for which saline was administered as placebo were used as negative controls, animals of the groups 1 and 2 were orally treated with the Paronychia kurdica extract at the doses of 5g and 10g only once respectively, those of the group 3 received a subcutaneous injection of the plant extract (5g) and those of the group 4 were treated every day for seven days by a percutaneous application of a pomade including 10% Paronychia kurdica. For oral and sub-cutaneous administrations, plant extracts were diluted to 50 mL with tap water. The application to teats was done separately and thoroughly for experimental and control groups.

MORPHOLOGICAL AND HISTOLOGICAL ANALYSIS

During the whole 90 day long experimental period, the treated and control animals were examined every 5 days and the characteristics of the warts were being compared with those of the pre-treated period. After a local anaesthesia (Prilokain; Citanest flk; Astra Zeneca), an injured tissue fragment was removed with scissors and formalin-fixed warts were embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin for evaluation by light microscopy [16].

The Paronychia kurdica extract was analysed by gas chromatographic-mass spectrometric method described of DENG and WILLIAM [10].

STATISTICAL ANALYSIS

The data were analyzed by One Way ANOVA, Post-hoc Tukey-HSD, the non parametric U Mann-Whitney test, and chi square test using SPSS (SPSS version 12.0 for Windows, SPSS Inc. Chicago, Illinois). Differences were considered as significant when P values were less than 0.05.

Results

The gas chromatographic-mass spectrometric method revealed important peaks corresponding to propanoic acid (90%), oleic acid (85%), linoleic acid (92%), α-tocopherol-acetate (72%), linol (82%) and linalool (86%) in wart weed.

Histopathological analysis revealed that the initial pathological lesions observed in the udder from the 30 cows were composed of 63.33% papilloma, 26.67% fibropapilloma and 10% fibroma (figure 1).

The initial wart numbers and the variations of the wart counts according to time and to treatment groups were reported in Table I. As the numbers of warts were highly heterogeneous between groups on day 0, the follow-up of treatments with Paronchia kurdica extracts has firstly consisted to compare the percentages of wart losses per animal during the whole experimental period (90 days) between groups as well as the proportions of completely cured cows per group and the time-point for which first disappearance of warts was recorded (Table II).

In controls (group 5), spontaneous regression of warts occurred since the 15° day in one cow. Thereafter, at the group level, wart losses slowly increased during the whole experimental period but remained low at the end of experiment (below 25%). In this group, only one animal exhibited

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FIGURE 1: Histological appearance of udder tumors. A. Papilloma of udder skin of a cow showing projections of hyperplastic epithelium (h) covered by a layer of keratin (k), haematoxylin-eosin, X 80. B. Fibropapilloma of udder skin of a cow showing fibromatous structure (f) and proliferated epidermis (h), covered by a layer of keratin (k), haematoxylin-eosin, X 120. C. Fibroma of udder skin of a cow showing loosely arranged fibromatous cells and fibres, haematoxylin-eosin, X 80.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
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<tbody>
<tr>
<td>Mean age (year)</td>
<td>4.3 ± 0.8 (3 ; 5)</td>
<td>4.7 ± 1.4 (3 ; 7)</td>
<td>4.0 ± 1.1 (2 ; 5)</td>
<td>4.7 ± 1.6 (3 ; 7)</td>
<td>4.0 ± 1.4 (2 ; 6)</td>
</tr>
<tr>
<td>Day 0: Initial count</td>
<td>15.3 ± 9.2 (7 ; 32)</td>
<td>36.8 ± 18.1 (7 ; 53)</td>
<td>26.5 ± 17.9 (12 ; 62)</td>
<td>13.5 ± 8.8 (5 ; 30)</td>
<td>29.2 ± 8.8 (18 ; 42)</td>
</tr>
<tr>
<td>Δwart (%) Day 5</td>
<td>0 ± 0% (0 ; 0)</td>
<td>-5.8 ± 14.2% (-34.8 ; 0)</td>
<td>0 ± 0% (0 ; 0)</td>
<td>0 ± 0% (0 ; 0)</td>
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<td>0 ± 0% (0 ; 0)</td>
<td>-9.4 ± 23.1% (-56.5 ; 0)</td>
<td>-16.8 ± 20.0% (-48.0 ; 0)</td>
<td>-1.0 ± 0% (0 ; 0)</td>
<td>-1.2 ± 3.0% (0 ; 0)</td>
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<td>-2.1 ± 5.1% (-12.5 ; 0)</td>
<td>-25.6 ± 30.2% (-69.6 ; 0)</td>
<td>-19.3 ± 23.5% (-60.0 ; -11.1)</td>
<td>-1.1 ± 2.7% (6.7 ; 0)</td>
<td>-7.4 ± 0%</td>
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<td></td>
<td>-41.6 ± 21.0% (-75.0 ; -11.1)</td>
<td>-52.4 ± 16.5% (-78.3 ; -28.6)</td>
<td>-28.0 ± 18.5% (-60.0 ; -11.1)</td>
<td>-1.1 ± 2.7% (-6.7 ; 0)</td>
<td>-7.4 ± 0%</td>
</tr>
<tr>
<td></td>
<td>-56.4 ± 26.8% (-87.5 ; -22.2)</td>
<td>-61.4 ± 14.3% (-79.2 ; -43.4)</td>
<td>-47.0 ± 22.5% (-72.2 ; -20.0)</td>
<td>-6.7 ± 8.4% (-7.2 ; 0)</td>
<td>-7.4 ± 0%</td>
</tr>
<tr>
<td></td>
<td>-61.2 ± 26.5% (-100.0 ; -33.3)</td>
<td>-83.3 ± 9.8% (-100.0 ; -71.7)</td>
<td>-57.7 ± 19.2% (-80.0 ; -25.0)</td>
<td>-7.8 ± 9.8% (-20.0 ; 0)</td>
<td>-5.4 ± 7.8% (-7.4 ; 0)</td>
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<tr>
<td></td>
<td>-67.0 ± 22.3% (-100.0 ; -38.9)</td>
<td>-93.1 ± 11.0% (-100.0 ; -71.7)</td>
<td>-76.4 ± 2.9% (-80.0 ; -25.0)</td>
<td>-11.6 ± 10.6% (-23.1 ; 0)</td>
<td>-8.2 ± 8.5% (-20.0 ; 0)</td>
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<td></td>
<td>-71.8 ± 21.8% (-100.0 ; -38.9)</td>
<td>-96.9 ± 7.7% (-100.0 ; -81.1)</td>
<td>-87.8 ± 9.0% (-100.0 ; -75.0)</td>
<td>-11.6 ± 10.6% (-23.1 ; 0)</td>
<td>-9.3 ± 8.4% (-20.0 ; 0)</td>
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<td>-80.1 ± 14.0% (-100.0 ; -58.3)</td>
<td>-96.9 ± 7.7% (-100.0 ; -81.1)</td>
<td>-96.4 ± 6.7% (-100.0 ; -83.3)</td>
<td>-13.3 ± 9.1% (-23.1 ; 0)</td>
<td>-14.0 ± 16.7% (-44.4 ; 0)</td>
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<tr>
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<td>-86.7 ± 12.4% (-100.0 ; -66.7)</td>
<td>-97.8 ± 5.4% (-100.0 ; -86.8)</td>
<td>-99.2 ± 2.0% (-100.0 ; -95.0)</td>
<td>-19.0 ± 10.6% (-30.8 ; 0)</td>
<td>-15.8 ± 20.9% (-55.6 ; 0)</td>
</tr>
<tr>
<td></td>
<td>-89.1 ± 13.5% (-100.0 ; -66.7)</td>
<td>-97.8 ± 5.4% (-100.0 ; -86.8)</td>
<td>-99.2 ± 2.0% (-100.0 ; -95.0)</td>
<td>-21.1 ± 6.7% (-30.8 ; 12.5)</td>
<td>-21.7 ± 31.3% (-83.3 ; 0)</td>
</tr>
<tr>
<td></td>
<td>-93.2 ± 8.0% (-100.0 ; -83.3)</td>
<td>-97.8 ± 5.4% (-100.0 ; -86.8)</td>
<td>-99.2 ± 2.0% (-100.0 ; -95.0)</td>
<td>-22.2 ± 8.1% (-33.3 ; -12.5)</td>
<td>-24.5 ± 37.9% (-100.0 ; 0)</td>
</tr>
</tbody>
</table>

Δwart: relative difference in wart counts for a given day calculated with the formula: 100(Wart number for a given day – initial wart number)/ initial wart number. Different superscripts a,b,c in the same line indicate significant differences (P < 0.05) between groups.

Table 1: Variations of wart counts observed on the teats of cows treated with wart weed per os (5g and 10g, groups 1 and 2), subcutaneously (5g, group 3) and every day for seven days with a pomade including 10% wart weed (group 4) or untreated (group 5) according to time (n = 6 per group). Results are expressed as mean ± standard deviation and extreme values are indicated into parenthesis.

a complete but delayed remission whereas 2 others did not show any papilloma regression. No significant difference in wart losses according to time was noticed between animals receiving the pomade including 10% of the plant extract (group 4) and controls, the profile of wart losses being similar in the both 2 groups. In addition, no complete cure was observed in the group 4. By contrast, wart losses were highly increased when the plant extracts were administered to cows orally (groups 1 and 2) or subcutaneously (group 3). They began at the 5th day in the group 2 (10g/day) or at the 10th day for the 2 other groups and they were significantly more marked in these groups compared to the controls and to the group 4 since the 15th day for the group 1 (5g per os) and since the 20th day for the groups 2 and 3. Wart losses became maximal...
in the group 2 from day 30 to day 60 (day 30: group 2 vs. group 3; \(P < 0.05\) and days 35 - 60: group 2 vs. group 1; \(P < 0.05\)). In this group, complete remissions in 5 cows were achieved between the 30th day (one case) and the 40th day (4 cases). In parallel, the wart disappearance intensity markedly increased on day 35 in the group 3 (5g, SC) and complete cure was obtained on Day 40 for one case and on Days 60 and 70 for 4 other cases, whereas in the group 1 (5g, per os), wart losses gradually increased in the same time and that total papilloma regressions in 3 cows were placed over Day 30 to Day 80. Between the 70th and the 90th days, wart losses were identical.

### Discussion

Although teat papillomatosis affected cattle usually younger than 2 years of age, all age classes may be concerned. In this study papillomas were recorded only in 2 - 7 years old animals and this is in agreement with data previously reported [21]. No agreement has been achieved on the methods used for the treatment of the disease [6, 20, 21]. These methods include cauterization, excision, cryotherapy, administration of local anaesthesia, autologous or heterologous vaccination, and autohaemotherapy. Alternatively, some drugs known to promote nonspecific stimulation of the immune system have reported to help papillomas regression, even to a complete disappearance were observed at the 5th – 10th days whereas in the control group, they were recorded later, on the 15th day. In addition, the numbers of complete remissions were significantly higher in these treated groups, reaching 3 to 5 cases among the 6 treated cows per group. Taking into account the percentages of wart losses, the proportions of total remission per group and their rapidity to occur, the oral administration of the plant extract at the dose of 10g appeared to be the most efficient treatment, following by subcutaneous injection of the Paronychia kurdica extract orally at 5g and 10g or subcutaneously (5g) compared to untreated controls. Furthermore, the first wart disappearances were observed at the 5th – 10th days whereas in the control group, they were recorded later, on the 15th day.

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**Table II**: Wart losses and regression cases observed on the teats of cows treated with wart weed per os (5g and 10g, groups 1 and 2), subcutaneously (5g, group 3) and every day for seven days with a pomade including 10% wart weed (group 4) or untreated (group 5) according to time (n = 6 per group).

<table>
<thead>
<tr>
<th>Wart losses (%)</th>
<th>Group 1 (5g, VO)</th>
<th>Group 2 (10g, VO)</th>
<th>Group 3 (5g, SC)</th>
<th>Group 4 (pomade)</th>
<th>Group 5 (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>Day 10</td>
<td>Day 5</td>
<td>Day 10</td>
<td>Day 15</td>
<td>Day 15</td>
</tr>
<tr>
<td>On day 90</td>
<td>93.2 ± 8.0 (^b)</td>
<td>97.8 ± 5.4 (^b)</td>
<td>99.2 ± 2.0 (^b)</td>
<td>-22.2 ± 8.1 (^a)</td>
<td>-24.5 ± 37.9 (^a)</td>
</tr>
</tbody>
</table>

**Remission**

<table>
<thead>
<tr>
<th>Absence</th>
<th>0 case</th>
<th>0 case</th>
<th>0 case</th>
<th>0 case</th>
<th>2 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>3 cases (^b)</td>
<td>5 cases (^b)</td>
<td>5 cases (^b)</td>
<td>0 case (^a)</td>
<td>1 case (^a)</td>
</tr>
<tr>
<td>dates</td>
<td>Day 30 (1 case)</td>
<td>Day 30 (1 case)</td>
<td>Day 40 (1 case)</td>
<td>Day 90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 70 (1 case)</td>
<td>Day 35 (2 cases)</td>
<td>Day 60 (3 cases)</td>
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</table>

Different superscripts \(^a\,\(^b\) in the same line indicate significant differences (\(P < 0.05\)) between groups (Pearson Chi-Square: 80,850).
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References


