Preliminary anti-tumour peptide therapy trial in bovine papillomavirus induced experimental hamster tumour model

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An experiment was conducted to study the preliminary therapeutic effects of anti-tumour WCS peptide in bovine papillomavirus (BPV-1&2)-induced hamster dermal fibroma tumour model. Gross and microscopic changes in peptide treatment at early interval fibroma indicated that growth was arrested while in well-grown tumours, effect of treatment was not much appreciable. Vascular changes, vacuolar degeneration, mononuclear cell infiltration and inflammatory changes were seen in tumour stroma. Ultrastructural features included presence of fibroblasts and fibrocytes, abundance of banded collagen fibres, enlarged pleomorphic nuclei, marked heterochromatin and abundance of rough endoplasmic reticulum. Certain nuclear and cytoplasmic organelle changes were indicative of therapeutic effects. It may be stated that anti-cancer WCS peptide had better therapeutic effects in early cases of experimentally-induced BPV tumours in hamsters.

Keywords: Anti-tumour therapy, bovine papillomavirus, hamster tumour model, pathology and ultrastructure, WCS peptide.

BOVINE papillomatosis (BP) is a common viral disease of the skin, mostly of young cattle, manifested as benign tumours or warts, caused by bovine papillomaviruses (BPVs). Papillomaviruses (PVs) are epitheliotropic and mucosotropic double-stranded DNA viruses that infect humans and cattle and, when associated with co-factors, can cause cancer and death. BPV is perhaps the most extensively studied animal PV. Six types of BPV have been identified: BPV-1, 2 and 5 causes cutaneous fibropapillomas, whereas BPV-3, 4 and 6 are responsible for squamous cell papillomas of the skin and oesophagus1. The BPV-2 has also been associated with urinary bladder neoplasia in enzootic bovine haematuria (EBH) of cattle2,3. It causes considerable economic impact on extensive cattle breeding worldwide4. BPVs are agents of diseases in farm animals and therefore of considerable veterinary and agricultural importance5.

Hamsters are nocturnal mouse-like animals with thick bodies, short tails and cheek pouches. The Syrian golden hamster (Mesocricetus auratus) is widely used in fundamental studies in virology and cancer research6 and is the animal of choice for transmission studies of BPV infection and produces fibromas7-10. Spontaneous atypical fibroma in thoraco-abdominal skin of aged male Djungarian hamsters are known to occur and also studied ultrastructurally. These tumours were composed of ganglion cell-like (GL) cells that had one or two ovoid nuclei, basophilic foamy cytoplasm, and various amount of collagen fibres between the cells. The tumour cells had abundant rough endoplasmic reticulum in the cytoplasm11. Peculiar subcutaneous abdominal skin tumour in Djungarian hamsters are also reported12. It is composed of proliferation of large spindle to polygonal cells that have abundant basophilic cytoplasm and a GL appearance accompanied by abundant interstitial collagen. Ultrastructurally, these cells contained abundant rough endoplasmic reticulum and Golgi complexes with dilated cisternae and intracellular collagen fibrils within these cisternae12.

A number of chemotherapeutic agents have been used for the cure of the cancer of various types in human and domestic animals including laboratory animals. Role of chemotherapeutic agents as anti-neoplastic agents is well known by various researches conducted recently. Synthetic peptides are organic compounds, generated by chemical approaches and are composed of two or more amino acids linked together by peptide bonds13. Tremendous advances in the development of methods for the synthesis of peptides lead to unique opportunities to apply designed synthetic peptide approaches to diverse areas. Epithelial tumours highly express COX-2 constitutively14. The anti-tumour activity of seven peptides including WCS (COX-2 inhibitors) was studied in vitro for 48 h in human oral carcinoma cell lines15. WCS peptide enters the nucleus and inhibits the function of COX-2, thereby retarding tumour progression. No information is available on its in vivo effects in any laboratory animal model.

Therefore, this study was planned with an objective to evaluate patho-morphologically anti-tumour therapeutic
effects of WCS peptide in the BPV-induced experimental hamster dermal tumour model.

**Materials and methods**

**Anti-cancer peptide (WCS)**

The anti-cancer WCS peptide was synthesized at the Department of Biophysics, All India Institute of Medical Sciences (AIIMS), New Delhi for experimental trial.16

**Experimental animals:** A total of 24 apparently healthy (12 male and 12 female), 6 weeks old, inbred Syrian golden hamsters (*M. auratus*) weighing about 70–90 g procured from the Division of Laboratory Animals, Central Drug Research Institute, Lucknow, Uttar Pradesh (UP) were used for experimental work. All the animals were maintained at Experimental Animal Sheds, Division of Pathology of this institute. Hamsters were housed in polystyrene cages. After acclimatization, they were weighed and randomly divided in three groups so as to give approximately equal initial group mean body weights. All the animals had free access to standard ration mixture, clean drinking water and examined daily for the health and husbandry conditions. Experiment was approved by the Institute’s Animal Ethics Committee.

**Preparation of infectious inoculum:** Cutaneous warts indicative of BPV-1 and -2 infection were sampled from different organized dairy farms of UP and Uttarakhand. Histopathologically confirmed cutaneous wart (fibropapilloma) samples were cleaned, washed, minced with sharp scissors and thoroughly triturated using sterilized pestle and mortar. A 10% homogenized suspension was prepared using phosphate-buffered saline (pH 7.2). The homogenized suspension was subjected to three cycles of freezing and thawing and centrifuged at 12,000 rpm for one hour. The supernatant was then filtered through a 0.45 μm syringe filter. Antibiotics were added to the supernatant and stored at –20°C till further use.

**Procedure of infection:** Animals of groups 2 and 3 were infected with 10% crude extract of warts by scarification on abdominal skin. First of all, hairs on abdomen were clipped with the help of a scissors. Scarification was done by a curved needle and then with a sterilized swab virus was applied at superficially wounded surface. In this way, infection was repeated thrice at 3 days interval.

**Experimental design:** It is given in Table 1. In another experiment, a total of six male animals were maintained for more than one year; four animals had tumour and two were control. The earlier four animals had pullet egg-like well-grown protruding tumours and these were used for mature or advanced case therapy.

**Treatment:** After 180 days, post-infection group 3 hamsters were treated with anti-cancer peptide. A total of 400 μg peptide was inoculated subcutaneously adjoining the tumour in two equal doses of 100 μg each at interval of two weeks along with 200 μg in the form of ointment after the last one week of S/C injection.

**Clinical observations:** Animals of all the groups were observed daily throughout the experimental period, for clinical signs and tumour growth. Body weight of the hamsters was recorded at monthly intervals.

**Haematological studies:** Approximately 1 ml of blood sample (two animals of each sex) from individual animals were collected from the heart on 120th day of the experiment into dry sterilized vial containing anticoagulant, ethylene diamine tetraacetic acid (EDTA) @1 mg/ml prior to sacrifice. Total leukocyte count (TLC) and differential leukocyte count (DLC) were determined.

**Serum biochemistry:** About 2.5 ml of blood from hamsters (two animals of each sex) were collected before sacrifice in test tubes for serum on 120 DPI. Sera samples were analysed for total protein, albumin and globulin using standard commercial kits (M/S Cogent). Value of total globulin was derived by calculation.

**Pathological studies:** Hamster tumours and skin biopsies from three animals on 15, 30 and 45 days post-treatment were taken surgically under local anaesthesia and biopsies were fixed in 10% formalin for histopathological examination. After proper fixation, tissues were cut into small sections with thickness of 2–3 mm, processed in ascending grades of alcohol for dehydration and cleared in benzene. The paraffin embedded tissues were cut into 4–5 micron-thick section and stained with haematoxylin and eosin. As and when required, the duplicate sections were stained with Van Giessen’s method for demonstration of collagen according to the conventional procedure.

**TEM studies:** For transmission electron microscope (TEM) studies, 1μm skin/tumour biopsies of hamsters (one animal each at 30 and 45 days post-treatment and control animals) were collected and tissue pieces were transferred to a petri dish containing few drops of chilled 2.5% gluteraldehyde in 0.2 M phosphate buffer (pH 7.4) for 6 h at 4°C. The tissue pieces were then washed with three changes (2 h each) of cold 0.2 M phosphate buffer (pH 7.4) and post fixed with 1% osmium tetroxide for 4 h at 4°C. The tissues were dehydrated in ethyl alcohol, cleared and embedded in epon-araldite resin. Ultra-thin sections (600 Å) were cut by employing ultramicrotome (Ultracut Reichert-Jung, Austria) and mounted on copper grids and stained with uranyl-acetate and subsequently by lead citrate. They were washed and allowed to dry on a
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Table 1. Design of experiment for anti-tumour therapeutic study in hamsters

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals and sex</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (NC)</td>
<td>8 (4M + 4F)</td>
<td>No infection with BPV and no treatment with peptide</td>
</tr>
<tr>
<td>Positive control tumour (PCT)</td>
<td>8 (4M + 4F)</td>
<td>Multiple scarifications (MS)/with cutaneous warts (CWs) (BPV-1 and -2)</td>
</tr>
<tr>
<td>Anti-cancer peptide (WCS) therapy (ACPT)</td>
<td>8 (4M + 4F)</td>
<td>MS with CWs (BPV-1 and -2) + treatment after 180 DPI*</td>
</tr>
</tbody>
</table>

M, Male; F, Female. WCS peptide was given at two-week intervals by S/C route and in the form of ointment as local application on abdominal skin tumour.

Table 2. Effect of ACPT on body weight of hamsters with dermal tumours

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6*</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>8</td>
<td>78.13 ± 3.15</td>
<td>89.00 ± 3.18</td>
<td>101.75 ± 3.28</td>
<td>113.75 ± 3.17</td>
<td>125.25 ± 3.23</td>
<td>137.00 ± 2.62</td>
<td>151.00 ± 2.24</td>
</tr>
<tr>
<td>PCT</td>
<td>8</td>
<td>73.00 ± 3.66</td>
<td>82.38 ± 3.19</td>
<td>92.38 ± 3.19</td>
<td>102.38 ± 3.19</td>
<td>112.50 ± 3.24</td>
<td>111.00 ± 4.33</td>
<td>134.25 ± 3.51</td>
</tr>
<tr>
<td>ACPT</td>
<td>8</td>
<td>91.50 ± 3.04</td>
<td>102.88 ± 3.17</td>
<td>112.88 ± 3.17</td>
<td>122.88 ± 3.17</td>
<td>132.88 ± 3.17</td>
<td>126.63 ± 2.46</td>
<td>136.38 ± 2.54</td>
</tr>
</tbody>
</table>

a, Significant difference from control group (P < 0.05);*On 6th month treatment was given to ACPT group.

Clinical observations

The observations in different groups were as follows:

Negative control (NC) group: No tumours were seen in any of the animals.

PCT tumour group: Numerous, nodular, lentil to pea-sized, raised, growths were seen on abdomen (Figure 1).

ACPT group: The observations of 30 days and one year were as follows:

30 days: Multiple, nodular, raised growths on abdomen were seen before treatment (Figure 1a). Suppressed tumour growths in all animals after WCS peptide therapy were observed (Figure 1b).

One year: Initially smaller multiple, raised nodular, pea to lentil size growth on abdomen was seen. Later arecanut-size raised whitish growths were observed on the abdomen. In one animal, two growths were bigger and protruded. Adjoining area showed necrosis and a smaller pea size growth. Raised nodular big size growths with superficial depressed and necrosed surface were also noted (Figure 1c and d).

Histopathology

The observations of 15, 30 and 45 days and one year were as follows:

15 days: Tumour revealed intact epidermis and the whorling pattern of haphazardly arranged proliferating...
fibroblasts. Cells were elongated and had hyperchromatic oval- or stellate-shaped nuclei. Blood vessels were engorged.

30 days: The epidermis was intact at places and below it, scantly or little to moderate reticulin fibres were seen. It was either band-like or mixed with tumour stroma or in abundance where there were no tumours with focal area of haemorrhages. Tumour was well grown and it had bundles of fibroblasts, fibrocytes and collagen fibres arranged in whirling pattern with presence of vacuolating spaces. Tumour was mature type and tumour stroma had bundles of fibroblasts, fibrocytes and collagen fibres arranged in whirling pattern with presence of vacuolating spaces. Tumour was mature type and tumour stroma had few lipocytes and at the base of the tumour, lipocytes were more prominent. Muscularis layer was more or less intact. Occasionally, at places in hypodermis or at the base of the tumour few mononuclear cell infiltrations were seen. In the other case, marked haemorrhages were found in tumour stroma and in muscularis. At the base of the tumour, blood vessels were seen engorged with intense infiltration of mononuclear cell and macrophages.

45 days: In this interval, intact epidermis along with its thinning and skin adnexae was seen. Reticulin fibres were prominent. Fibromatous tumour stroma was transparent and light pinkish colour along with cystic spaces and vacuolations. More lipocytes were present in hypodermis. Blood capillaries were engorged. In the other case, the tumour was found compact and mature with more lipocytes. Eosinophilic stained reticulin fibres were numerous (Figure 2).

One year: The findings of both cases were as below:

Case no. 1: At the periphery of tumour, epidermis was intact at some places, whereas in other areas it was degenerated and necrosed depending upon underlying changes in tumour stroma. Tumour consisted of fibroblasts, arranged in different patterns. Fibroblasts had oval to elongated nuclei with tendency of nuclear crowding. In some other areas, tumour stroma showed vacuolations and blood capillaries were engorged. At the periphery of superficial surface, tumour was necrosed and in this area, cells were not distinct and epidermis also showed degenerative changes.

Case no. 2: On the superficial surface of tumour, epidermis was intact. At some places slight cornification, focal necrosis and suppuration was seen. It showed tendency of epidermal hyperplasia and presence of few vacuolated cells. In hypodermis, a layer of fibrocytes with some fatty vacuoles was observed. It also had hypertrophied sebaceous glands and other skin adnexae. Blood capillaries were highly engorged between hypodermis and at periphery of the tumourous growth.

At the periphery, a zone of growing tumour with whirling patterns of fibroblasts and fibrocytes were observed. It had stellate-shaped hyperchromatic nuclei. This was followed by homogenous eosinophilic area rich in collagen. Tumour stroma was studded with engorged blood capillaries and vessels. At deeper places, necrosed areas with dead and degenerating cells and without structural details was observed. The tumour was diagnosed as fibroma with regressive changes (Figure 3).

Ultrastructural studies

The TEM features were as follows:

Control group: Skin comprised epidermis and hypodermis. In toluidine blue-stained light microscopy (LM) sections, epidermis appeared thin and hypodermis contained...
lipocytes characterized by vacuoles of ring-like structure. The epidermis had elongated/elliptical to somewhat oval cells with plenty of cytoplasm. Nuclei of these cells had uneven borders with marked perinuclear zone of heterochromatin. Nucleolus was missing in areas examined but in one cell it was centrally placed. Intercellular spaces were marked and numerous microvilli were seen on uneven indistinct cytoplasmic membrane. Cytoplasm was electron dense and organelles were not distinct. Banded collagen was in abundance in adjoining cells. Hypodermis had capillaries with typical endocytes and pericytes. Its lumina had plasma and erythrocytes. The nuclei of these cells were similar to the ones in epidermis with marked heterochromatin. The wall of lipocytes was ring-shaped. It had fibre-like structure and lysosomes.

**ACPT group**

The TEM features were as follows:

*30 days:* Tumour was comprised fibroblasts/fibrocytes which were elongated/stellate shaped. Cytoplasmic...
processes were very long in some cells. The intercellular space between cells was plenty and it was filled with banded collagens which were in close vicinity of tumour cells. Nuclei of tumour cells were enlarged with altering nucleo-cytoplasmic ratio. In most of the cells, heterochromatin was prominent. The nuclear membrane was even or uneven in some cells. Occasionally, it was bizarre-shaped. Nucleoli were not seen in any area of tumour cells examined. Size of cross-section of collagen fibre was 46–71 nm. Cytoplasm was scanty and electron dense as such only few organelles were discernible. These included rough endoplasmic reticulum (RER) and mitochondria. Cytoplasmic membrane was uneven (Figure 4).

One year: Ultrastructurally fibroma comprised numerous fibroblasts and fibrocytes along with long tapering
cytoplasmic processes. Intercellular space was pronounced and filled with numerous banded collagen fibres surrounding the cells. Longitudinal or cross-section of these collagen fibres was observed. Microvilli were another feature of intercellular areas which were arising from cytoplasmic membrane. Tumour cell nuclei were large, pleomorphic (elongated, oval and bizarre shaped) with prominent peri-nuclear heterochromatin, peripheral margination/marginated nucleolus. Occasionally, binucleolus were also discernable. Enlarged nuclei also caused change in nucleo-cytoplasmic ratio. Cytoplasm was electron dense with abundance of dilated cisterns of RER, degranulated RER and numerous banded collagen fibres. These collagen fibres were electron dense and of 49 to 56 nm in size on cross-section. Cytoplasm also contained few large vacuoles, numerous variable sized electron dense lipid droplets and mitochondria with indistinct cristae. Certain vacuoles were filled with oedematous fluid (Figure 5).
Discussion

An experiment was conducted to study therapeutic effects of a new WCS anti-cancer peptide on BPV-induced hamster dermal tumour laboratory model. For this anti-cancer peptide therapy was clinicopathologically studied in search of a new drug.

Increasing trend of body weight was seen in all groups during pre- and post-experiment period which was indicative of progressive growth of hamsters with benign tumours. No significant differences in body weight were seen in different groups post treatment. TLC count was significantly decreased in PCT and increased in ACPT groups as compared to controls. Similarly, DLC count showed significantly increase in monocytes in ACPT group as compared to others. Serum biochemistry revealed increased value of globulin in ACPT. These changes are indicative of humoral and cell-mediated
status against BPV-induced tumours in hamsters. Further literature is scanty on above parameters in experimental dermal fibroma induced by BPV in hamsters\textsuperscript{20,21}.

Tremendous advances in the development of methods for the synthesis of peptides led to unique opportunities to apply designed synthetic peptide approaches to diverse areas\textsuperscript{22}. Clinically, multiple tumour growths were arrested on the abdomen in ACPT group between 15–45 days of treatment. Histopathologically, in ACPT group after 15 days of treatment, suppressed tumour growths were
observed. Morphological changes associated with therapy included engorged blood vessels. After 30 days, fibroma showed vacuolating spaces and at the base of tumour lipocytes were more prominent. Occasionally, at places in hypodermis or at the base of tumour few mononuclear cell infiltrations were seen. In another case, marked haemorrhages were found in tumour stroma and in muscularis. After 45 days, intact, thin epidermis and skin adnexae were seen. Reticulin fibres were prominent. Fibromatous tumour stroma was transparent and light pinkish in colour along with cystic spaces and vacuolations. Eosinophilic stained reticulin fibres were numerous. From our observations it may be interpreted that tumour growth were arrested and further it failed to grow progressively. In this group, no adverse clinical reaction or any mortality occurred. These findings are in partial accordance with earlier worker who found more or less similar changes in CW-induced tumour treated with chicken infectious anaemia (CIA) pcDNA-VP3 plasmid. Such changes were not reported in previous studies in the untreated, experimentally-induced CW tumours in hamsters.

Our in vivo observations are in accordance with earlier workers who reported that WCS peptide showed anti-tumour activity in vitro on human oral carcinoma cell lines. It was observed that WCS peptide had more anti-tumour activity than others as it inhibited the proliferation of tumour cells. WCS peptide has been identified as a tripeptide inhibitor which is a potential lead for a new class of COX-2 inhibitor. The screening of this peptide by enzyme linked immuno absorbent assay (ELISA) test showed that WCS inhibits COX-2 by more than 85% and preventing the reaction of substrate arachidonic acid with the enzyme supports the possibility of peptide WCS as potent and competitive inhibitor of COX-2 (ref. 15).

COX-2 is not detectable in normal tissues and is induced by cytokines, growth factors, oncogenes and tumour promoters. COX-2 overexpression is reported in many epithelial neoplasms, including human, rat, canine and cultured urothelial cancer cells. COX-2 derived PGE2 synthesis may contribute to tumour cell resistance to apoptosis and hence to tumour development and progression. Further, COX-2 could increase tumour invasiveness with activation of the matrix metalloproteinase MMP-2. Previous studies have also demonstrated that H-ras-transformed cancer cells (human breast cancer cell lines and rat intestinal epithelial cells) over express COX-2 (refs 33, 34). COX-1 and COX-2 expression in 20 urothelial carcinomas of the urinary bladder in cows suffering from chronic enzootic haematuria induced by BPV-2 using immuno-histochemical methods was reported . COX-2 was expressed in 17 of 20 urothelial carcinomas. Moderate to intense COX-2 labelling was detected in both non-invasive and invasive urothelial carcinomas. These authors concluded that COX-2 is overexpressed in naturally occurring urothelial carcinomas of cows. Since CWs/EBH are caused by BPV-1&2, WCS peptide needs to be further explored for its therapeutic purpose in early tumour growths in bovines too.

However, in case of mature tumour; raised, big, nodular growths with superficial depressed and necrosed surface were noted. Vacuolations, engorgement, necrosis at the periphery of superior surface and indistinct cells were noted with degeneration in epidermis. In the other case of mature tumour, focal necrosis and suppuration was seen. In hypodermis, engorgement between hypodermis and at periphery of the tumourous growth was noted. In advance grown tumours encouraging therapeutic results were not observed. Possibly dose of WCS peptide was not optimum and it needs to be further standardized.

Electron microscopic studies

In toluidine blue-stained LM sections, normal skin epidermis appeared thin and hypodermis contained lipocytes characterized by vacuoles of ring-like structures. TEM showed elongated/elliptical to somewhat oval cells with plenty of cytoplasm. Nuclei of these cells had uneven borders with marked perinuclear zone of heterochromatin. Intercellular spaces were marked and numerous microvilli were seen on uneven, indistinct cytoplasmic membrane. Cytoplasm was electron dense and organelles were not distinct. Banded collagen was in abundance adjoining to the cells. The wall of lipocytes was ring-shaped. It had fibrill-like structures and lysosomes. The TEM features of skin are in accordance with that of Djungarian hamsters. Ultrastructural features of early and mature fibroma in different groups included presence of fibroblasts and fibrocytes, banded collagen fibres, enlarged pleomorphic nuclei, marked heterochromatin, scanty cytoplasm, altered nucleo-cytoplasmic ratio, presence of RER and mitochondria. Occasionally, cytoplasmic vacuoles and microvilli were also observed. It is difficult to comment on the effect of therapy on ultrastructural features of different stages and different groups of dermal fibroma. However, some of these nuclear and cytoplasmic features were indicative of degenerating and dying cells due to the effect of WCS peptide.

Similar ultrastructural features of fibroma and related tumours were described earlier also including few reports in hamsters. Ultrastructural features of fibrohistiocytic tumours were described. This revealed that fibrosarcomas and fibrous histiocytomas contain cells with a spectrum of differentiation, ranging from a fibroblastic appearance, to others with myoid properties. Although myofibroblasts may predominate, fibrosarcomas usually contain a greater proportion of fibroblasts. Histioctyloid cells, fibroblasts, myofibroblasts, giant and foam cells, and to a lesser extent myoid (smooth muscle-like) cells.
characterize fibrous histiocytomas and malignant fibrous histiocytomas.

Diagnostic criteria of fibrous neoplasms were described as abundant rough endoplasmic reticulum and type 1 (banded) collagen closely surrounding the cells. It showed that fibroblasts also had a prominent Golgi apparatus and they may or may not have filopodia and long, tapering polar processes. Lipid vacuoles in varying numbers may also be present in the cytoplasm. Fibrosarcoma show intimate admixture of spindle-shaped cells and collagenous matrix, irregular shape of some of the nuclei as a criterion for malignancy. The cytoplasm is rich in dilated endoplasmic reticulum, long cytoplasmic processes, microfilaments and high nucleo-cytoplasmic ratio.

In this study, certain ultrastructural features such as bizarre-shape nuclei, margination of nucleoli, margination and condensation of heterochromatin, electron dense cytoplasm and certain organellae may be suggestive of apoptosis and dying tumour cells. In the case of apoptotic death, the cell nucleus is early and specifically involved. TEM study showed a chromatin margination, followed by its compactness towards the nuclear periphery, to form one or several frequently cup-shaped masses. The nucleus appeared markedly rearranged when compared to the normal one, which shows a perinuclear and a perinucleolar dense heterochromatin, clearly distinguishable from the diffuse interchromatin.

Further, it is reported that apoptosis progresses with an increase in the number of perichromatin granules and perichromatin fibrils, a reduction in number of interchromatin granule centres concomitant with an increase in their size, partial digestion and circumferential condensation of the DNA at the nuclear membrane and rounding of the cytoplasm with an increase in organelle density and shrinkage in cell size. Seven oesophageal squamous carcinomas, treated with pre-operative chemotherapy (mitomycin-C, ifosfamide and cisplatin-MIC) were examined by electron microscopy. Tumours showed cytotoxic damage in that apoptosis and unusual necrotic changes were observed in almost all the neoplastic cells.

From this preliminary study, it may be stated that anti-cancer WCS peptide had better therapeutic effects in early cases of experimentally induced BPV tumours in hamsters. More studies are required using other molecular tools to support observations.


ACKNOWLEDGEMENTS. We thank ICAR-NAE Project for funding this research and K.P. thank the Institute for Junior IVRI Fellowship. We are also thank the Director, IVRI, Izatnagar, UP for all necessary facilities.

Received 19 February 2010; revised accepted 20 December 2010