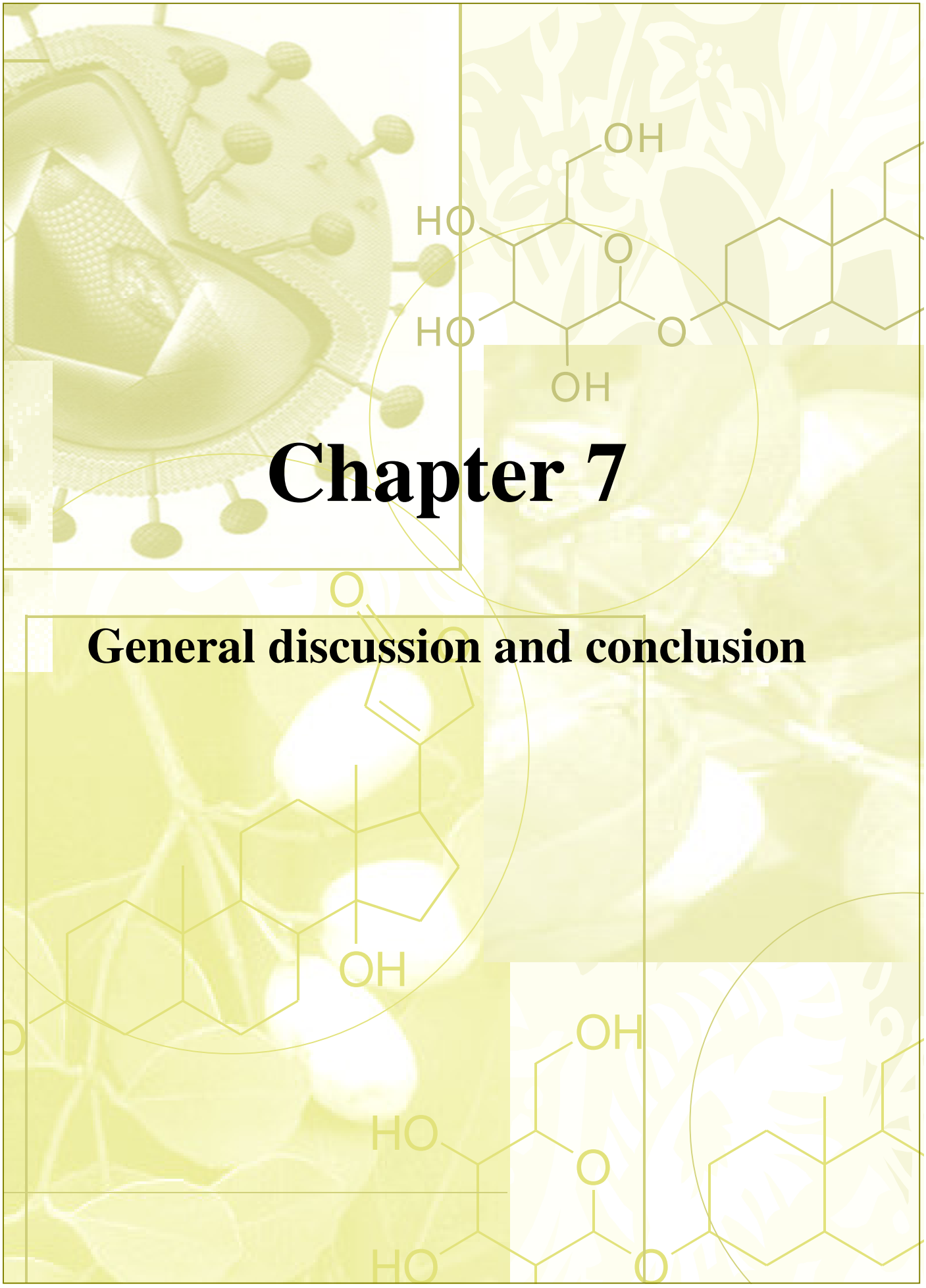

Chapter 7

General discussion and conclusion

Since the discovery of HIV in 1981, 25 years ago, there has not been any solution to the ever-growing disease. Many vaccine trials have not resulted in any usable information, and still we have no method to kill the virus, and to help the people already suffering from the disease. Current medicines are only selectively effective, and the prevalence- and death rate of the disease increase every year in South Africa. Very little progress has been made in any of the related fields of HIV. Currently there is very little hope that the disease will be contained and overcome before it is too late.

South Africans are still ignorant about the infection and prevalence rate of HIV in the country. South Africa has the highest rate of infection in the world, and more than 5 million of the population are already infected with the virus. The mid-2005 population of South Africa is estimated at 46.9 million. The latest results available for South Africa at the end of 2003 indicated that 5.3 million had already been infected with the virus. The overall estimate of HIV-prevalence rate is approximately 10%, while the prevalence rate for adults aged 15-49 is estimated at 17%. When this is compared to the prevalence rate of Sub-Saharan Africa of 7.4%, South Africa compare badly to other sub-Saharan countries (Statistics South Africa, 2002).

A cure for HIV is definitely not within reach for a few years to come. Research contributed very little to understanding the mechanism and progress of the virus during infection. The need for an effective drug to contain the virus becomes more evident as more people are realising that they are dying from AIDS. This does not only have a social implication for families, but an enormous economic impact on every level of the country as well.

In this study twelve extracts were prepared and tested against HIV. Only one of the extracts showed inhibition of the virus. All the extracts were however potent anti-microbial extracts, and shows that anti-microbial activity is therefore not an accurate indicator of HIV activity. The extract prepared from the leaves of the indigenous tree *Elaeodendron croceum*, showed promising results. The extract was purified and from the extract a very active pure compound had been isolated. The crude extract was tested first for antiviral activity against HIV, with an inhibiting activity of the VSV-pseudotype assay of more than 80%. Silica gel columns and liquid-liquid fractionation were used to obtain a semi-purified extract. The semi-purified extract had comparable activity to the crude extract with inhibition of approximately 85%. The chloroform extract is easily prepared from the crude extract, and the stable compounds at room temperature make the preparation of the extract easy and usable in any type of environment.

The chloroform extract was purified further by column chromatography using silica gel columns. To obtain the pure compound, fractions containing the compound were identified by TLC, and after evaporation of the last solvents, the pure compound precipitated and formed white crystals.

To identify the compound it was necessary to use several analytical methods. NMR was the most useful method to determine the structure and position of the substituents on the structure. For confirmation of the positions of the substituents, it was necessary to use two-dimensional NMR. The purified active compound had been identified by these methods as digitoxigenin-glucoside. The compound yielded even

better results on the anti-HIV assays than was found with the crude and chloroform extracts. The compound inhibited the virus in the HeLa-Tat-Luc assay with 85% and the recombinant virus with approximately 90%.

Toxicity tests were performed to determine if the compounds could be used on living cells without having high cell toxicity. *Elaeodendron* spp. are known for their toxic compounds, and it was therefore expected that this plant extract would be toxic as well. Traditional healers often use toxic compounds as medicine. It is important to ensure the safety of these compounds taken, by controlling the dosages of the intake.

The extract and the pure compound of *E. croceum* were not toxic as expected. The pure compound was more toxic than the crude- and the semi-purified extracts, but still not very toxic. The toxicity did increase rapidly when the concentration of the semi-purified extract was increased. The active concentration of 100 ng/ml for digitoxigenin-glucoside was much lower than the toxic concentration of 25 µg/ml. The chloroform extract was less toxic than the pure compound at 100 ng/ml, and it only became toxic at concentrations of 50 µg/ml. These in vitro results seem to indicate that the extract and pure compound from *E. croceum* are not as toxic as expected. These results are however on in vitro tests only and need to be confirmed by in vivo results as well.

The isolated compound belongs to the extended group of cardiac glycosides. The structure is derived from digitoxigenin which is similar in structure to digitoxin and digoxin. All these compounds are very toxic, but they are also used medicinally because of their cardiotonic activity. Extensive research was done on the group of

cardiac glycosides determining their effectivity and toxicity when used as a cardiotonic. Several derivatives and variants were produced to decrease the toxicity but still keep their cardiotonic potency (Rathore *et al.,* 1985), (Humber *et al.,* 1983) & (Takiura *et al.,* 1974).

Digitoxigenin was tested for its cardiotonic and Na^+/K^+-ATPase activity. It was found that the sugar attachment improves the binding ability of the compound and it increased the activity of this compound (Rathore *et al.,* 1985). Digitoxigenin-glucoside only contains one glucose moiety in comparison to the three sugar moieties of digoxin and digitoxin. The orientation of the glycoside also determines its activity. β-D-glycosides such as the compound isolated were more effective than the α-D-glycosides. Most of the naturally occurring glycosides exhibit the β-D-orientation, as found in digitoxigenin-glucoside.

The type and number of sugar molecules are also correlated with the toxicity of the cardiac glycosides. Less sugar molecules decrease the toxicity of the compound, but it has a negative influence on the potency of the compound. Digitoxigenin-glucoside is therefore less toxic than most of the cardiac glycosides that contain more than one sugar molecule.

In terms of cardiac activity, a less potent compound would not be useful, although it would be less toxic than other similar compounds. Other applications of the less toxic cardiac glycosides like the isolated compound are therefore worth considering.

No previous antiviral activity had been shown for digitoxigenin-glucoside or any of the related cardiac glycosides. This is therefore the first report of the cardiac glycosides being active against viruses, and specifically HIV. As the compound was very active against the virus in the VSV-pseudotype assay using a recombinant virus, it is possible that digitoxigenin-glucoside could inhibit the virus directly. This assay focuses on the viral structure and not on enzymes and enzyme products responsible for the survival of the virus. The decreased toxicity is a big advantage, as the concentration of the compound can still be increased significantly before the start of toxicity to the cells.

This compound is also different from anti-retroviral treatment (ART) used at present that blocks or inhibits some of the viral enzymes or enzyme products. These medicines cannot eliminate the virus from the body, but can only reduce the viral load in the blood. Medicines used today are only useful to keep a person in the asymptomatic phase, and prolongs the development into AIDS with the progress of other opportunistic infections (OI). Digitoxigenin-glucoside is unique in its activity targeting the virus itself, and the activity is probably not because of its toxicity, but because of a specific interaction with the virus.

As the glucose moiety is responsible for attachment in the heart, the glucose might also play a role in attachment to the virus. The active concentration of 100 ng/ml is more than 250 times less than the toxic concentration of 25 µg/ml determined on the VERO cells. It can therefore potentially be a very useful drug in combination with current ART to lower the viral load, but also eliminating the virus from the blood stream. More *in vivo* experiments are however necessary to determine its activity.

There would always be the problem with the viral DNA that is incorporated into the host DNA. The DNA would stay in the cells, and it would be ideal to eliminate the virus before it enters the nucleus to be integrated into the genome.

Considering the prospects of this compound to be used as a medicine, there are still some obstacles to overcome. Once the virus enters the nucleus, there is no stopping the replication process of the virus, as it will always be part of the genome. Prevention is still however, better than a cure, as the drug will only lower the viral load by eliminating the virus from the blood system. This will in itself reduce the risk of transmitting the virus to another person. It would still be better to prevent the virus from infecting the cells, than to lower the viral load and to eliminate the virus from the blood stream.

A medicine to eliminate the virus from the blood stream would be a great advantage to the drugs that are currently in use. It would increase and strengthen the efficacy of ART. As the compound could be synthesised in a laboratory, harvesting of the plant would not be necessary, and the species would not be endangered.

Further research and funding could pave the way for digitoxigen-glucoside or related cardiac glycosides to reach the markets as ART drugs. Although new medicines could reach the market, prevention will still play the biggest role in combating the virus from depleting the work force of our country.