Lemon and Lime juice as potent natural microbicides

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Background:-

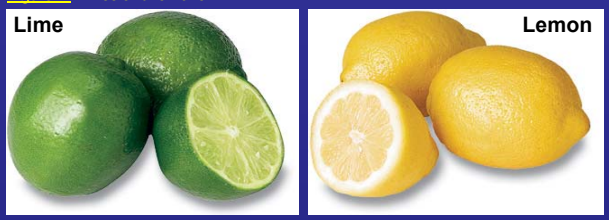
There are estimated to be 5 million new HIV infections per year with more women than men now becoming infected. This highlights the desperate need for a cheap, readily available, female controlled microbicide. Intravaginal lemon juice applied prior to intercourse has been used as a contraceptive by women around the Mediterranean for more than 300 years. We have confirmed the contraceptive properties of lemon juice by showing that a 20% final concentration of lemon juice in fresh human ejaculate irreversibly immobilises 100% of sperm in under 30 seconds. Even today, intravaginal lemon and lime juice douches are used by women in Nigeria to protect themselves from pregnancy and supposedly from sexually transmitted infections. Furthermore, we have shown that daily intravaginal administration of neat lime juice to macaque monkeys for one month causes no vaginal pathology, and similar small scale safety studies in humans are planned. But lemon and lime juice may do much more than just immobilise sperm.

The human immunodeficiency virus, HIV, is inactivated by acidic pH. The purpose of our study has therefore been to determine the effectiveness of lime and lemon juice as a microbicide when added to HIV *in vitro*, and to human ejaculates containing HIV.

Methods:-

Sterile pools of lemon juice (pH 2.3) and lime juice (pH 2.4) were prepared by filtration. Live cultures of HIV-BaL were exposed to neat lime or lemon juice to give final concentrations in the range 0-20%, and the pH of the mixture was determined. The cultures were examined 2, 5 and 60 minutes after addition of the juice and the presence of live virus was determined by it's ability to infect mitogen-activated peripheral blood mononuclear cells (PBMC), which were then cultured for 14 days. Viral replication was measured indirectly by viral reverse transcriptase activity in culture supernatants. Toxicity of lemon and lime juice to the PBMC's was measured by trypan blue exclusion and 14C leucine incorporation. Finally, seminal plasma was spiked with 100,000 copies of HIV-NL4.3, and then treated with 0-20% lemon juice for 5 minutes. Viral load was measured by reverse transcriptase activity.

Figure 1: Limes and lemons



Results:-

Citrus juice concentrations in the range of 5-20% greatly reduced the pH of the culture medium, and were toxic to >90% PBMC's.

Table 1: Lemon and lime juice have similar pH at different dilutions in media.

	% (v/v)	0	2	5	10	15	20
pH	Lemon juice	7.8	5.7	4.0	3.4	3.0	2.9
	Lime juice	7.7	6.0	4.3	3.7	3.2	2.9

We therefore developed a revised system in which HIV-1 was exposed to lemon or lime juice for 2-60 minutes. After incubation, the citrus juice was diluted out with media prior to adding to PBMC's for long term culture. Using this modification, over 60% of PBMC's were still viable after 24 hours, even at the highest concentrations of citrus juice (20% for up to 60 minutes).

Table 2: Diluted concentrations of lemon and lime juice in PBMC cultures show that concentrations of 0-15% are not toxic to the cells as tested by trypan blue exclusion.

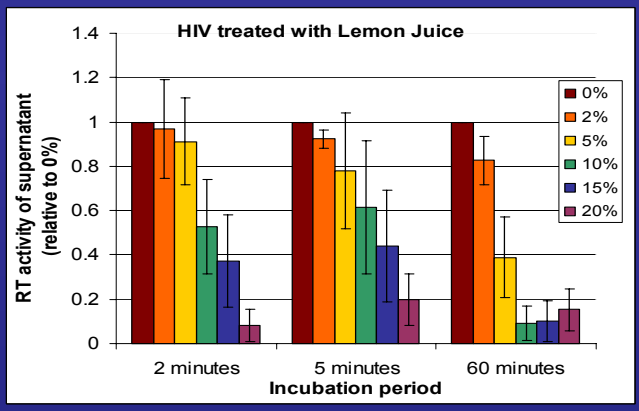
Concentration of juice (% v/v)	Initial*	0	2	5	10	15	20
	Final*	0	0.2	0.5	1.1	1.8	2.5
pH after dilution	Lemon	7.9	7.3	7.1	6.5	6.0	5.3
	Lime	7.8	7.2	72	6.7	6.1	5.6
Proportion viable cells (%)	Lemon	79	80	78	77	74	62
	Lime	75	75	78	78	72	63

*Initial concentration used to treat HIV-1; * Final concentration after dilution in medium

Once we accounted for toxicity to PBMC's, we tested the microbicidal effect of lemon and lime juice on live HIV.

In culture, a 20% concentration of lemon juice inactivates 80% of HIV in as little as 2 minutes, whilst 10% lemon juice takes up to 60 minutes for the same effect. A 5% dilution of lemon juice can inactivate half of the added virus within 1 hour of exposure. These results were mirrored when lime juice was used.

Figure 2: Inhibition of viral replication after incubation with varying dilutions of lemon juice. Figures are expressed relative to our positive control (0%).



Preliminary results on 4 spiked ejaculates indicate that lemon juice inactivates HIV in ejaculate after just 5 minutes, although the effect appears to be less pronounced than that observed in our culture system.

Table 3: RT assay results for spiked semen treated with lemon juice (cpm/uL)

Lemon Juice dilution	Sample 1	Sample 2	Sample 3	Sample 4
0%	327.5	184	178.5	136.5
10%	242	111.5	100.5	128
20%	160.5	47	186	100.5

Conclusion:-

Lime or lemon juice added to cultures of HIV has the ability to inactivate the virus. Lemon juice added directly to PBMC's was toxic at concentrations of >5% over a week in culture. The protocol was altered so that lemon juice was initially incubated with HIV for up to 60 minutes and then diluted to non-toxic concentrations prior to adding to PBMC's. A 20% concentration of lemon juice in culture medium inactivated 80% of HIV after 2 minutes, whilst a 10% concentration takes up to 60 minutes for the same effect. Preliminary results suggest a similar effect on HIV in ejaculate. The microbicidal properties of lemon and lime juice are likely due to the low pH produced by citric acid. Citric acid is also a major component of the normal human ejaculate. Further laboratory testing and clinical trials are required before lemon or lime juice can be recommended as a means of preventing transmission of HIV-1.