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A novel mechanism of action prevents ethanol-induced injury

Liv.52: Profile of an herbal remedy

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Liv.52 was introduced in 1954 as a specially formulated Ayurvedic herbal remedy for the treatment of viral hepatitis, which had assumed epidemic proportions in Delhi and other metropolitan cities in India. The remedy was found to be generally useful and has been widely prescribed for infective hepatitis since then.[1,2] During the next 25 years, beneficial effects of Liv.52 have been reported in various hepatic disorders.[1-10] Experimentally, Liv.52 was demonstrated to prevent injurious effects of carbon tetrachloride and other toxic substances on the liver. Clinically, stimulation of appetite and an increase in serum albumin concentration were consistently seen. These effects were particularly prominent chronic alcohol users.

Liv.52 is an Ayurvedic formulation available as tablets and syrup containing the following herbs: *Capparis spinosa; Cichorium intybus; Solanum nigrum; Terminalia arjuna; Cassia occidentalis, Achillea millefolium; Tamarix galica* and *Phyllanthus amarus*. These herbs are processed and formulated according to the principles of Ayurveda, which are aimed at enhancing efficacy and avoiding toxicity.[11]

The safety of Liv.52 has been demonstrated by acute and chronic toxicity studies on animals and a phase 1 safety study in human volunteers. During the past 38 years, Liv.52 has been used by millions of patients in India and Europe and no adverse effects have been reported. The uniformity of the product is ensured by using authentic herbal material and by rigidly following the formulation processes according to the principles of Ayurveda. The finished product has been standardised by thin layer chromatographic finger printing. A recent demonstration of its effects on ethanol metabolism has led to the development of bioassay. This ensures that the end product has uniform biological activity.

The following is an account of the objectively demonstrable biological effects of Liv.52 and their clinical significance.

Ethanol - induced impairment of liver function: Effect of Liv.52

Ethyl alcohol is widely regarded as an hepatotoxic agent. The toxic effects of ethanol are believed to be due to the accumulation of acetaldehyde - an intermediary metabolite of ethanol.[12] Though fatty liver formation and development of liver cirrhosis are known, adverse effects of ethanol, a measurable acute effect on liver function in human being was first demonstrated by Harshe.[13] He and his colleagues measured the uptake of intravenously administered radiolabeled Rose Bengal (RBI^{131}) using the whole-body profile scanner and body segment counter. They demonstrated that consumption of 120 ml of whisky in 60 minutes significantly depressed RBI^{131} uptake within two hours. Administration of Liv.52 for two weeks prevented this effect of ethanol ingestion. Placebo administration did not influence the depression of RBI^{131} uptake caused by ethanol (Fig. 1).

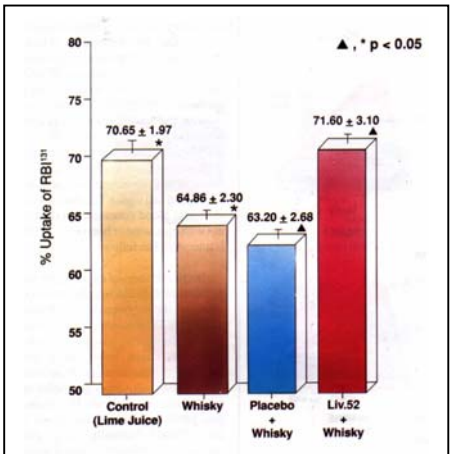

Fig. 1. Hepatic RBI^{131} uptake at 25 minutes after i.v. injection

It was then necessary to study the effects of Liv.52 on the metabolism of ethanol and acetaldehyde. Lower ethanol and higher acetaldehyde concentrations were reported in chronic ethanol users.[14] Acetaldehyde was measured by head space gas chromatography, but the method was susceptible to artifacts.[15] Chauhan and Kulkarni[16] overcame the artifacts by collection of blood in chilled perchloric acid, followed by immediate incubation and measurement. This enabled them to measure blood concentrations of ethanol and acetaldehyde and to study the effects of Liv.52. They confirmed that regular users of alcohol had lower blood ethanol concentrations. The possible mechanisms responsible for this change and the consequences of acetaldehyde accumulation are depicted in Figure 2.

Administration of Liv.52 to chronic alcohol users caused elevation of blood ethanol levels and also an initial increase in blood acetaldehyde levels followed by rapid decline.[17] This was interpreted to signify the inhibition of the presystemic metabolism of ethanol (Chronic ingestion of ethanol causes induction of enzymes playing a role in ethanol-metabolism). In the same study, it was shown that Liv.52 treatment enhanced the urinary excretion of acetaldehyde. This was interpreted to mean that the binding of acetaldehyde to cell proteins (especially in the liver) was prevented by the administration of Liv.52. This rapid elimination of acetaldehyde from the body may be responsible for the protective effects of Liv.52 in alcoholic liver disease.

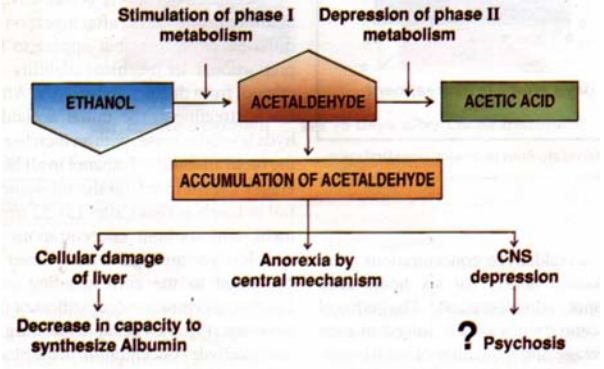

Fig. 2: Ethanol metabolism in regular alcohol users

Acetaldehyde accumulation is known to cause unpleasant symptoms referable to the central nervous system. This was the basis for using disulfiram to dissuade users of alcohol. It is quite likely that symptoms of hangover felt by heavy drinkers may be due to high acetaldehyde levels persisting for longer times. Chauhan and Kulkarni[18] evaluated cognitive functions after a standard drinking session before and after treatment with Liv.52 for two weeks. Cognitive functions showed less impairment after Liv.52 treatment, suggesting that the rapid elimination of acetaldehyde caused by Liv.52 may be responsible for this effect.

Different alcoholic beverages and effect of Liv.52

It is well know that blending of ethanol differs in different beverages and consumers show preferences for a particular beverage not only for its taste and aroma, but for the effect on mood. Interesting differences have been observed by Chauhan and Kulkarni in the absorption of ethanol from six different beverages.[19]

Six commonly used alcoholic beverages (i.e. whisky, gin, vodka, rum, beer and wine) were administered to six volunteers on six different occasions separated by 48 hours. Blood ethanol and acetaldehyde concentrations were measured hourly for six hours after ethanol administration. The ethanol concentration was determined in each beverage and a quantity of each beverage containing 43 gm of ethanol was to be ingested in one hours. The study was repeated after administration of Liv.52 for two weeks. The results were very interesting and demonstrated separate effects of Liv.52 on ethanol absorption and acetaldehyde elimination. Firstly, the ethanol concentration in blood from the same amount of alcohol varied with the beverages. Whisky showed the same levels as in previous studies, but rum showed significantly higher levels - similar to those seen in non-users of ethanol - while beer showed the lowest levels, indicating poor bioavailability. The effect of Liv.52 also varied with the beverage. With whisky, there was a predictable increase in blood concentration,

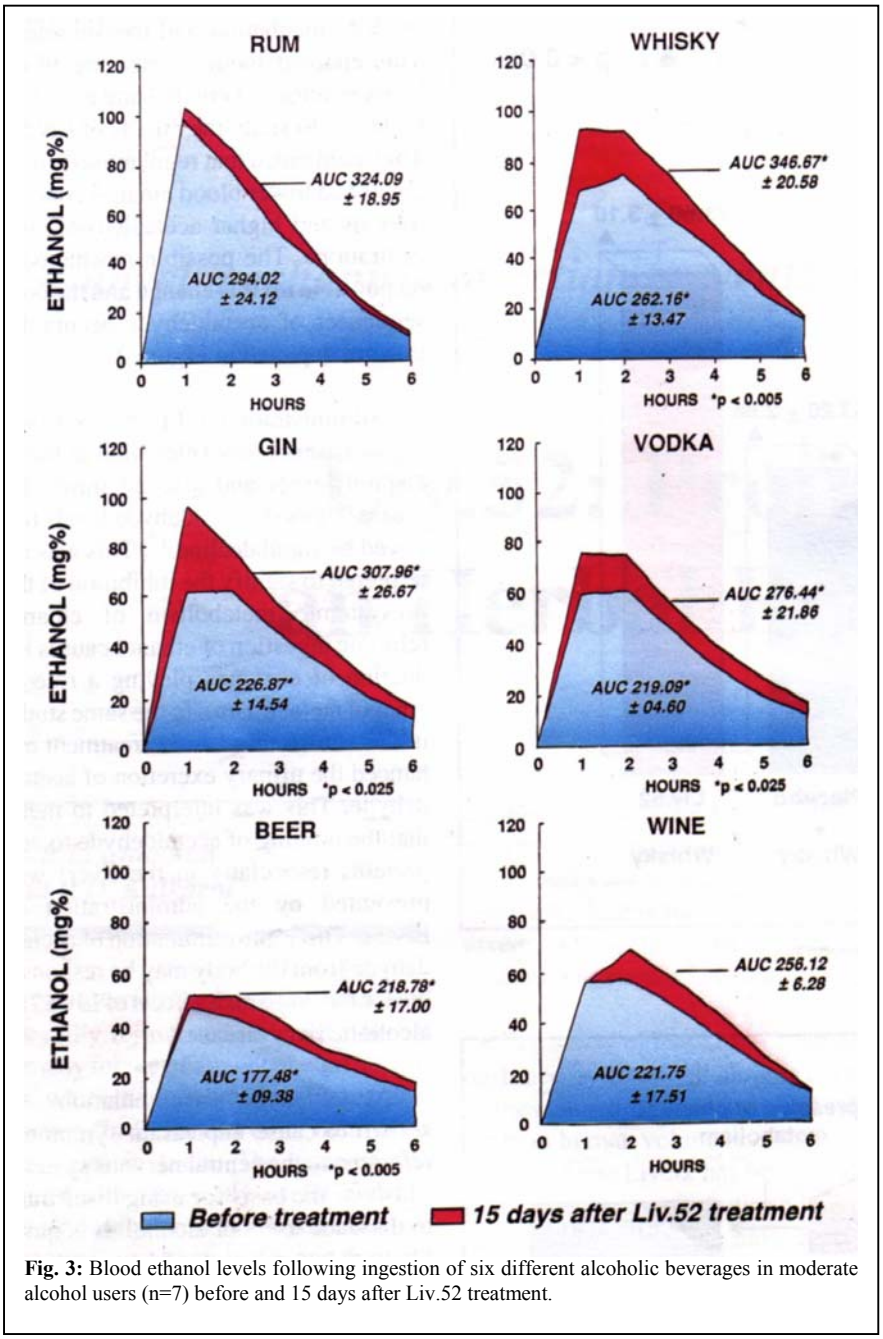

Fig. 3: Blood ethanol levels following ingestion of six different alcoholic beverages in moderate alcohol users (n=7) before and 15 days after Liv.52 treatment.

and also so with gin and vodka. But there was no change in blood concentration of ethanol with rum, wine or beer (Fig. 3). This is interpreted as follows:

In chronic users of ethanol, low levels of ethanol are due to induction of enzymes of the presystemic metabolish, while Liv.52 enhances blood levels by inhibiting presystemic metabolism – the effect being more pronounced in chronic users. Rum probably contains substances that inhibit the presystemic metabolism and ethanol, thus producing high ethanol levels in chronic users. Naturally, Liv.52 does not further enhance the ethanol levels. Beer probably has substances that interfere with ethanol absorption, resulting in a poor bioavailability not improved by Liv.52.

Acetaldehyde levels before Liv.52 treatment are different after ingestion of different beverages, but appear to be proportional to the bioavailability of ethanol from different beverages. After Liv.52 treatment, the initial acetaldehyde levels increase, with an increase in the bio-availability of ethanol in all beverages except rum. But the subsequent fall in levels is faster after Liv.52 treatment, and six-hour concentrations of acetaldehyde are significantly lower as compared to the corresponding pre-Liv.52 concentrations, regardless of the beverage (Fig. 4). The rapid lowering of acetaldehyde concentration in the blood is reflected in higher excretion of acetaldehyde in the urine over a six-hour period (Fig.5).

Serum albumin and Liv.52

Early clinical reports mentioned an increase in serum albumin in patients to liver cirrhosis has been established.[24] Liv.52 by its local effect on the intestine, probably inhibits ethanol-metabolising enzymes locally and causes an increase in the bioavailability of ethanol. Herbal drugs have been shown to inhibit presystemic metabolism of other drugs and to increase their bioavailability.[25] The effect of increasing the bioavailability of ethanol may result in reduced ethanol intake, which may additionally protect the liver and also be economically beneficial.

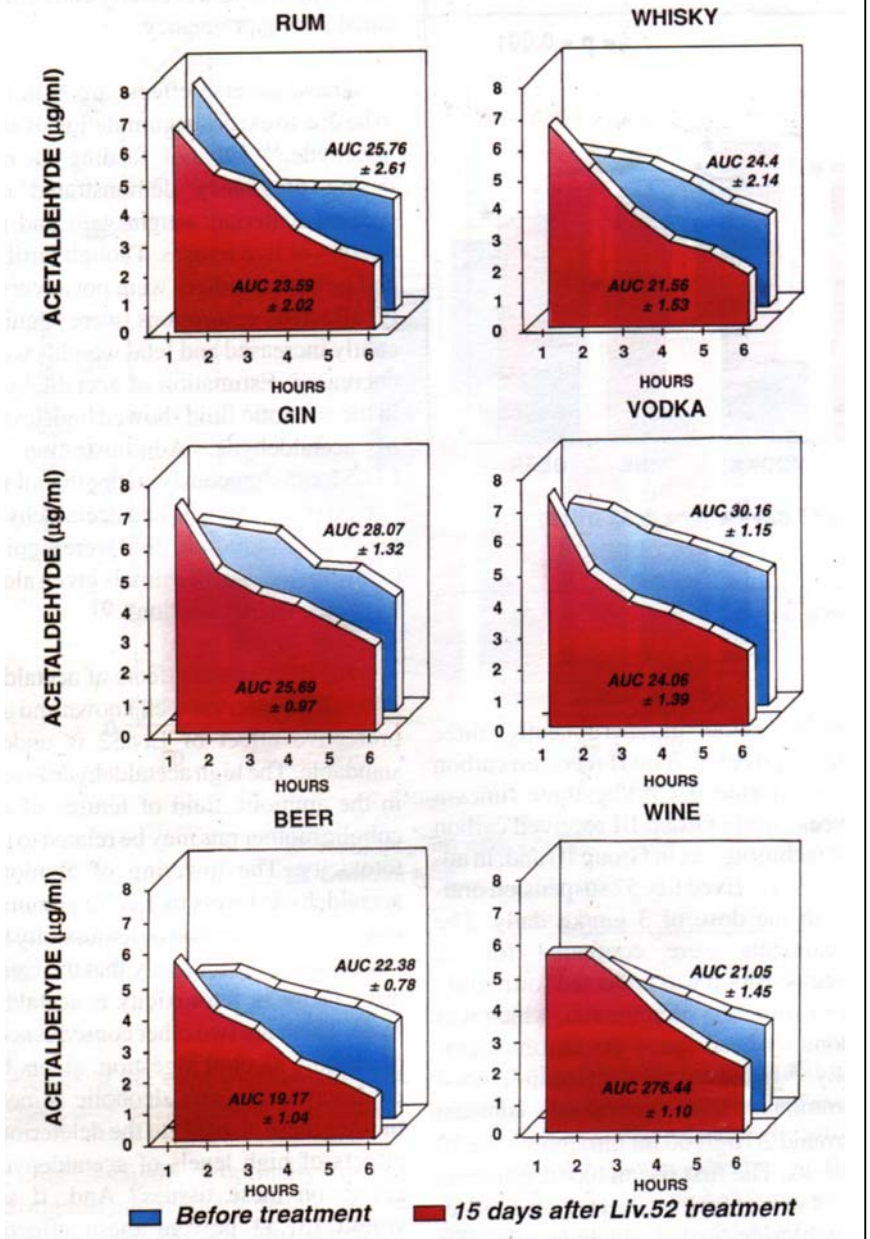

Fig. 4: Blood acetaldehyde levels following ingestion of six different alcoholic beverages in moderate alcohol users (n=7) before and after Liv.52 treatment.

Hepatic encephalopathy - blood ammonia and Liv.52

Beneficial effects of Liv.52 have been clinically seen in fulminant cases of viral hepatitis.[26] One of the consequences of the failure of parenchymal cell function is inability to convert ammonia into urea and the consequent rise in blood ammonia levels, which are correlated with symptoms of hepatic encephalopathy[27] Hepatic encephalopathy can be produced in rats by administration of carbon tetrachloride for prolonged periods.

We tried to study the effect of Liv.52 on carbon tetrachloride-induced hyperammonemia. Rats were divided into three groups. Group I received 0.2ml/kg saline intraperitoneally three times a week. Group II received carbon tetrachloride 0.2 ml/kg three times a week, while Group III received carbon tetrachloride (as in Group II) and, in addition, received Liv.52 suspension orally in the dose of 3 gm/kg daily. The treatments were continued for 12 weeks. Blood was collected fortnightly for estimation of ammonia, which was done by head space gas chromatography.[28] In the animals of Group I, blood ammonia level remained constant around 20 ug/100 ml throughout the 12 weeks. The first rise in blood ammonia was seen at four weeks in the carbon tetrachloride-treated animals. At this time, the Liv.52-treated animals did not show any rise in blood ammonia vis-a-vis the control group animals. At eight weeks, the blood ammonia levels in the carbon tetrachloride-treated groups were three times as high as those in the control group and remained so till the 12th week. In the Liv.52-treated group, the blood ammonia levels were significantly lower at all estimation points (Fig. 6).

Fetotoxicity of ethanol and Liv.52

The adverse effects of maternal alcohol consumption on foetal development are well documented.[29,30] Even moderate drinking is clearly contraindicated during pregnancy.

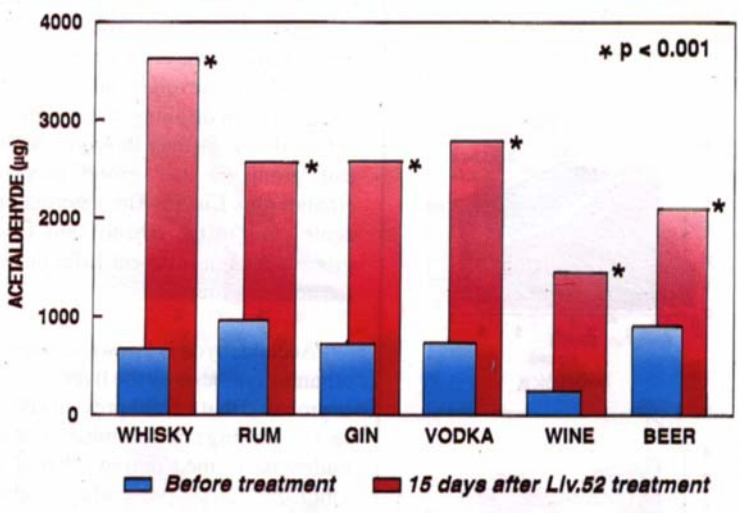

Fig. 5: Mean urine acetaldehyde excretion (mcg), 0-6 hours.

These adverse effects are believed to be due to excess accumulation of acetaldehyde.[31] Ethanol feeding to rats during pregnancy demonstrated decreased maternal weight gain and reduction of live foetuses. Though fertility and gestation indices were not adversely affected, resorptions were significantly increased and foetal weights were decreased. Estimation of acetaldehyde in the amniotic fluid showed high levels of acetaldehyde. Administration of Liv.52 simultaneously with ethanol reversed these changes and acetaldehyde levels in the amniotic fluid were significantly lower than in animals given alcohol alone (in publication).

The deleterious effects of acetaldehyde on the liver are well known and the protective effect of Liv.52 is understandable. The high acetaldehyde levels in the amniotic fluid of foetuses of alcoholic mother rats may be related to foetotoxicity. The lowering of amniotic acetaldehyde levels by Liv.52 administration and prevention of foetotoxicity by Liv.52 also make it likely that the causative factor in foetotoxicity is acetaldehyde. There are two other consequences of chronic alcohol ingestion: alcoholic cardiomyopathy and alcoholic dementia. Are these also due to the deleterious effects of high levels of acetaldehyde acting on these tissues? And, if so, would Liv.52 prevent these effects? These aspects of ethanol toxicity and the effect of Liv.52 are at present being actively investigated by us.

Non-alcoholic liver diseases and Liv.52

In alcohol-induced impairment of liver function, the mechanism of the preventive action of Liv.52 can be explained by its action in causing rapid elimination of acetaldehyde. Can its beneficial effects in non-alcoholic cirrhosis and non-alcohol-related liver diseases, like viral hepatitis and drug-induced liver damage, be explained on the basis of the same mechanism? The answer may well be in the affirmative.

Acetaldehyde is normally formed during the intermediary metabolism of fatty acids and glucose. This is quickly converted to acetic acid and enters the tricarboxylic acid cycle. Even so, small changes in blood acetaldehyde levels (which are so far not accurately measurable) caused by food may play a physiological role in the appetite satiety rhythm. Acetaldehyde is known to have central nervous system effects that can be demonstrated with disulfiram ethanol interaction. In minute quantities, acetaldehyde may suppress appetite selectively. Larger amounts of acetaldehyde formed during a fatty meal may be responsible for termination food intake altogether. When the functional mass of parenchymal cells is reduced due to any cause such as hepatitis, cirrhosis or drug toxicity, acetaldehyde metabolism may be the first to suffer, causing elevation of acetaldehyde concentration in the blood. This appears probable because anorexia is the earliest and most constant symptom of liver dysfunction and improvement in liver function is heralded by the return of appetite.

Accumulation of acetaldehyde has been shown to have a cytotoxic effect on liver cells[32] and to inhibit its own metabolism, establishing a vicious circle. Thus, in any parenchymal disease of the liver - viral, bacterial, parasitic, drug-induced or idiopathic - accumulation of acetaldehyde and aggravation of cellular damage will occur. In fact, in many conditions, the effects of acetaldehyde

may be more harmful than the original disease itself. It is, therefore, not surprising that Liv.52 has been shown to be beneficial in many hepatic disorders of varying etiology.

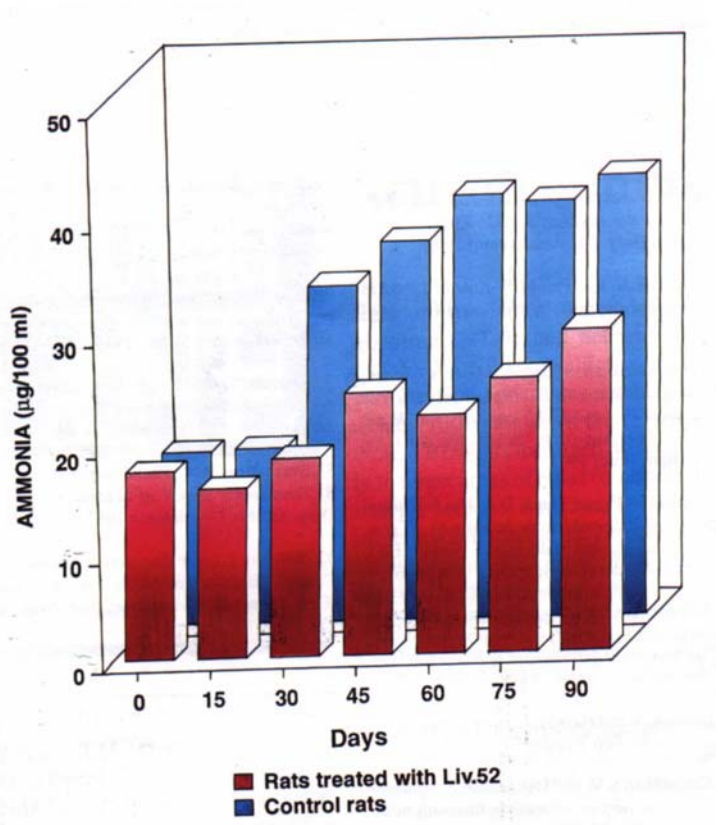

Fig. 6: Blood ammonia levels in CCl_4-induced hyperammonemia in rats and the effect of Liv.52

CONCLUSIONS

Liv.52 is apparently a crude herbal preparation. But it must be stated that the formulation is carefully processed according to pharmaceutical processes described in Ayurvedic texts and in keeping with Ayurvedic principles of formulation. These are quite different from modern pharmaceutical and chemical processes, but there is no doubt that the processes are aimed at concentrating the active principles in the most bioavailable form, though not in a chemically pure form. It is now being increasingly realised that the formulation may have biological properties different from the active ingredients contained in it. The so-called inert additives do modify the biological behaviour of pure chemicals, especially regarding their kinetic properties. Therefore, as long as uniformity of the formulation is assured and biologic effect is demonstrated, modern medicine should have no hesitation in accepting this useful remedy.

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