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Protective Effect Of An Indigenous Drug Livomyn On Ketoconazole Induced Hepatotoxicity

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Livomyn, an indigenous drug, was studied against hepatic damage induced by the antimycotic drug, ketoconazole. Serum levels of transaminases - alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase and lipids (cholesterol and triglycerides) were used as the biochemical markers of hepatotoxicity. The results indicate that, in albino rats, Livomyn showed protective effect against ketoconazole- induced hepatotoxicity and consequent liver damage.

SEVERAL substituted imidazole derivatives are currently available for the treatment of variety of fungal infections,[1,2]. Ketoconazole, a substituted imidazole, is used as an antifungal agent, it has been reported to have a broad spectrum activity against systemic and superficial mycoses, and is effective when administered orally,[3,4,5,6].

However, the hepatotoxic effect of ketoconazole has been reported by several investigators.[7,8,9] The aim of study was to evaluate the role of an indigenous drug, Livomyn for its protective action against ketoconazole-induced hepatotoxicity and consequent liver damage. It was also decided to investigate the duration and extent of ketoconazole-induced hepatotoxicity.

MATERIALS AND METHODS

Livomyn. comprising 20 different plant products (**Table-1**) in syrup base, was provided by Charak pharmaceuticals, Bombay. The syrup base without the medicinal ingredients was used as a placebo. Ketoconazole used in the present study was purchased from commercial sources. Carboxy methyl cellulose (CMC) was obtained from Sigma, U.S.A.

Albino male rats weighing between 120 to 180 g were procured from Haffkine Institute, Parel, Bombay, and were maintained for a week on photoperiod in the Central Animal Facility at 30°C at relative humidity of 50 to 80%. They were fed chow (National Food Supplier, Bombay), tap water, **ad libitum** and were housed in rat cages. Six group of rats, eight per group, were kept in seperate cages, each cage holding two from a group.

TREATMENT: After a week of aclimatization the following treatment schedule was employed. All administrations were done through oral route once a day, as shown in **Table.2**.

CMC was used for making ketoconazole suspension. Therefore, to rule out hepatotoxicity of its own, a group of rats were fed only CMC and this group was included as one of the control group. Since Livomyn has a syrupy base, it was felt essential to keep syrup as a control group. One group of control rats received Livomyn only.

Serum analyses : Serum ALT and AST were estimated by the colorimetric method of Reitman

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Table 1 : Composition of Livomyn (Ayurvedic preparation)

Sr. No.	Botanical Name	Common Name	Quantity of aqueous extract / 5 ml.
1.	**Eclipta alba**	Bringraj	250 mg.
2.	**Coriandrum sativum**	Dhania	250 mg.
3.	**Cocculus cordifolius**	Gudchuli	500 mg.
4.	**Rosa domascena**	Gulabphul	250 mg.
5.	**Chrysantheum coronarium**	Guldavri	250 mg.
6.	**Solanum dulcamara**	Kakmachi	250 mg.
7.	**Ocimum bacilicum**	Kalitulsi	250 mg.
8.	**Andrographis paniculata**	Kalmegh	250 mg.
9.	**Gynocardia odorata**	Kadachhal	500 mg.
10.	**Cissampeles parera**	Kalipath	250 mg.
11.	**Lawsonia alba**	Mendipan	250 mg.
12.	**Ipomoeg turpethem**	Nishottar	250 mg.
13.	**Pipe longum**	Pipli	250 mg.
14.	**Fumaria officialis**	Pittapapda	250 mg.
15.	**Boerhaavia diffusa**	Punarnava	500 mg.
16.	**Rhamnus wightii**	Raktarodhida	250 mg.
17.	**Cascara esculenta**	Saptarangi	250 mg.
18.	**Tephrosia purpurea**	Sharapankha	250 mg.
19.	**Zingiber officinale**	Sunthi	250 mg.
20.	**Embelia ribes**	Vavading	500 mg.

and Frankel.[10] Following incubation of serum with appropriate buffer, serum ALT catalyses the following reaction :-

$\propto$ Ketoglutarate + L - Alanine $\rightleftharpoons$ L-Glutamate + Pyruvate .....(A)

Similarly serum AST catalyses the following reaction :-

$\propto$ Ketoglutarate + L-Aspartate $\rightleftharpoons$ L-Glutamate + Oxaloacetate ....(B)

The resulting products of A and B when coupled with 2,4-DNPH gives a brown colored complex which was read colorimetrically at 505 nm.

Serum alkaline phosphatase was estimated by colorimetric method of king and Armstrong[11] The serum and substrate when incubated with a buffer

Table 2 : Treatment Schedule

Group	Name of Drug	Daily dose	Duration
I.	Control (C.M.C.)	1 ml/100g. of body weight	Till the end of study
II.	Control (Placebo Syrup)	1 ml/100g. of body weight	Till the end of study
III.	Control (Livomyn alone)	1 ml/100g. of body weight	Till the end of study
IV.	Ketoconazole (Single dose treatment)	20 mg/100g. body weight	For one day only
V.	Ketoconazole	20 mg/100g. of body weight	For 2, 4 and 6 weeks
VI.	Ketoconazole + Livomyn	20 mg/100g. + 1 ml/100g. of body weight	for 2,4 and 6 weeks

Table showing the drugs, their daily dosage and duration of treatment in rats.
The doses of ketoconazole and Livomyn were selected five times the human dose.

of pH between 910 results in the hydrolysis of the substrate liberating p-nitrophenol, which at an alkaline pH is an yellow coloured complex. This was read colorimetrically at 510 nm.

Serum cholesterol was determined by the colorimetric method of zuokowsky.[12] Cholesterol in the serum reacts with acetic anhydride in the presence of glacial acetic acid and concentrated sulphuric acid to form a green coloured complex. Intensity of the colour is proportional to the cholesterol concentration and were measured at 575 nm.

Serum triglycerides were estimated by colorimetric method of Fletcher[13] and Bucolo.[14] In this method serum triglycesides were extracted with isopropanol. Triglycerides so extracted were saponified with alcoholic potassium hydroxide liberating glycerol. Glycerol was oxidised with periodate to produce formaldehyde. This inturn was made to react with acetyl acetone in the presence of ammonium ions to produce yellow coloured diacetylhydrotoludine which was read at 415 nm.

Statistical analysis was performed using students 't' test.

RESULTS

Administration of CMC, syrup base or livomyn alone did not affect liver function. The average values of all the parameters were identical, indicating that these agents by themselves have not adverse effect on hepatic function.

Administration of ketoconazole in a single dose of 20 mg/100 g of body weight orally failed to induce appreciable liver damage at the end of 24 hrs, when compared with control values. However, administration of ketoconazole in a dose of 20mg/100 g of body weight for two, four or six weeks induced appreciable liver damage as indicated by the elevated levels of serum transaminase (ALT and AST), alkaline phosphatase and lipids (cholesterol and triglycerides). (Fig. 1,2,3,4 and 5)

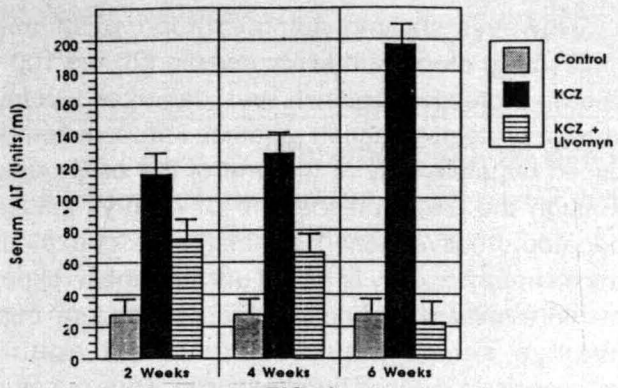

FIG. 1

Serum ALT levels in ketoconazole and Ketoconazole + Livomyn treated rats. Histogram shows change in mean serum ALT levels after oral administration of ketoconozole alone and its combination with livomyn during 6 weeks Results are mean ± S.D.

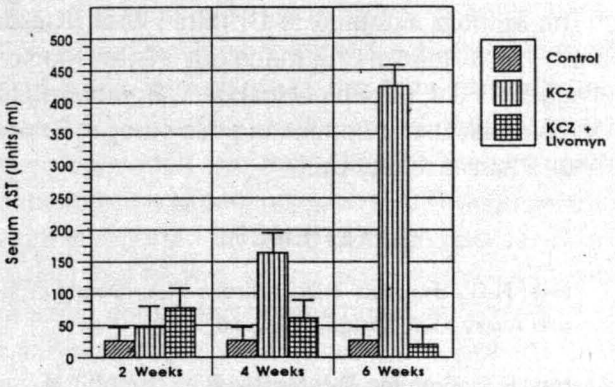

FIG. 2

Serum AST levels in ketoconazole and Ketoconazole + Livomyn treated rats. Histogram shows change in mean serum AST levels after oral administration of ketoconozole alone and its combination with livomyn during 6 weeks Results are mean ± S.D.

Further, administration of ketoconazole (20 mg/100 g of body weight) and subsequent treatment with Livomyn (1 ml/100 g of body weight) orally for two, four or six weeks showed protection against liver damage as evidenced by lowering of serum ALT, AST, alkaline phosphatase, cholesterol and triglycerides, when compared with values obtained from rats treated with ketoconazole (20 mg/100 g of body weight) alone for the same period. (Fig. 1,2,3,4 and 5).

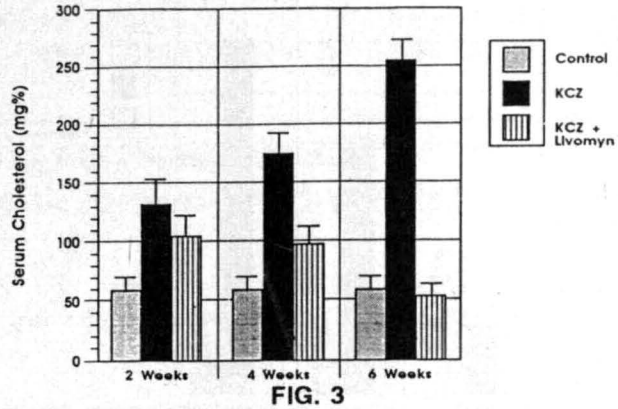

FIG. 3

Serum Cholesterol levels in ketoconazole and ketoconazole + Livomyn treated rats. Histogram shows change in mean serum Cholesterol levels after oral administration of Ketoconozole alone and its combination with livomyn during 6 weeks Results are mean ± S.D.

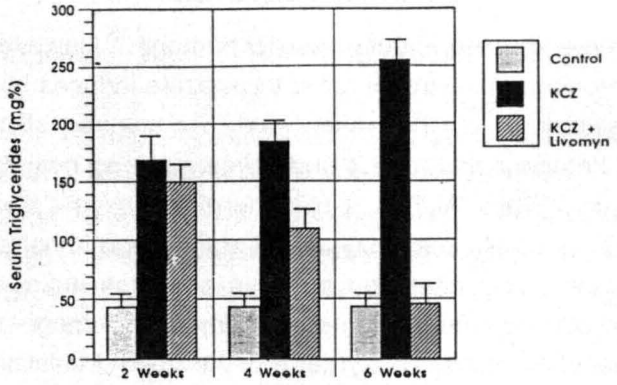

FIG. 4

Serum Triglyceride levels in ketoconazole and Ketoconazole + Livomyn treated rats. Histogram shows change in mean serum Triglyceride levels after oral administration of Ketoconozole alone and its combination with livomyn during 6 weeks Results are mean ± S.D.

DISCUSSION

The present investigation revealed that acute administration of ketoconazole (single dose treatment) fails to induce appreciable live damage. Clinical studies also do not show hepatic injury after acute administration of ketoconazole.[15] It is reported that certain species of animals shows hepatic injury, but only when ketoconazole is administered in a dose that exceeds by several-fold the maximum rec-

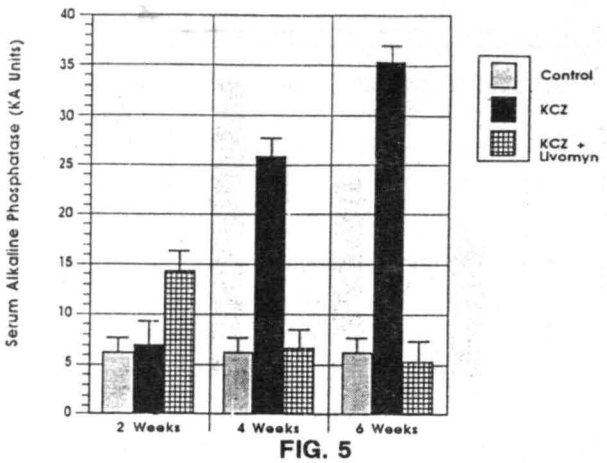

FIG. 5

Serum Alkaline Phosphatase levels in ketoconazole and ketoconazole + Livomyn treated rats. Histogram shows change in mean serum Alkaline Phosphatase levels after oral administration of Ketoconozole alone and its combination with livomyn during 6 weeks Results are mean ± S.D.

ommended therapeutic dose for humans.[15] However chronic administration of ketoconazole induces hepatic injury.[16] In the present study oral administration of ketoconazole for 2,4 and 6 weeks shows hepatic injury. Since ALT is a mitochondrial enzyme and AST is a cytosolic enzyme, primarily found in hepatocytes, any alteration in these enzymes indicate the altered membrane permeability. The damage of liver cells releases enzymes in to the blood circulation and consequently levels in blood found to be elevated than normal.

The elevation of alkaline phosphatase indicates the disturbed excretery function of liver. It is reported that ketoconazole impairs the bile flow and biliary bile acid output.[17] Serum lipids, cholesterol and triglycerides show rising pattern at the end of 2,4 and 6 weeks of ketoconazole treatment. Rise in lipid levels indicates depression of esterification in parenchymal cells of liver. As a consequence of such hepatic damage induced by ketoconazole, rise in free cholesterol levels in the serum are enhanced. It is reported that ketoconazole inhibits the metabolism of cholesterol by inhibiting the cytochrome-P-450[17,18].

However chronic administration of livomyn (1ml/100 g) along with ketoconazole (20 mg/100 g) shows protection against liver damage. Livomyn shows protective action against ketoconazole-induced hepatotoxicity at the end of 2,4 or 6 weeks. Though the exact mechanism of livomyn-induced hepatoprotective action against ketoconazole-induced hepatotoxicity is as yet unclear, these experimental results clearly indicate that on long term basis livomyn shows a protective effect against ketoconazole induced hepatotoxicity. Thus our study shows that an indigenous reparation of livomyn protects the animal against ketoconazole hepatotoxicity. Though we have not studied the precise mechanism of action of livomyn, it could be similar to the one reported with other hepatotoxins.[19]

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