

## **A Controlled Trial of Choleric and Hepatoprotective Actions of Livzon and Dehydrocholic Acid in a Model of Obstructive Jaundice in Albino Rats**

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RATAN, J., ROHATGI, S., GUPTA, D.K. and RATAN, S. *A Controlled Trial of Choleric and Hepatoprotective Actions of Livzon and Dehydrocholic Acid in a Model of Obstructive Jaundice in Albino Rats.* Tohoku J. Exp. Med., 1997, **181** (1), 161-166 — The authors have tried to examine the hepatoprotective and choleric action of a new indigenised drug, Livzon (Hind Chemicals Ltd., Kanpur, India) and compared its action to Decholin (casella—Riedel Pharma GmbH, Frankfurt, Germany), a known hepatoprotective and choleric agent. Albino rats were chosen as the experimental animals. Obstructive jaundice was created by ligating the common bile ducts after taking liver biopsies. The animals were divided into three groups: (i) Control group-no drug was given, (ii) Livzon trial group, (iii) Decholin group. The animals were reoperated, liver biopsies were taken and histologically examined. The study confirmed the hepatoprotective and choleric actions of Livzon and Decholin. However, Decholin was more of a choleric, the Livzon was more hepatoprotective. ——— biliary obstruction; hepatoprotective; choleric; Decholin; Livzon

The role of medical management of obstructive cholangiopathy is limited as surgery has become feasible for most of these disorders. However, Knodell and Holloway (1976), Kitani et al. (1977), Di Padova et al. (1980) and Dumont et al. (1980) found that addition of choleric and cholagogues brings a promising effect to the medical management of obstructed biliary tree with hepatocellular damage in experimental animals.

Many studies have been done by several authors like Hoeffler et al. (1987), Wang et al. (1987), Okuno et al. (1988) and Lexa et al. (1989) in this respect with

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medicines obtained from various plants or their extracts. A new Ayurvedic drug "Livzon" has been recently marketed in India which has been claimed to have the choleretic and the hepatoprotective actions (data collected from the research laboratory, Jammu, Jammu and Kashmir, India). Though dehydrocholic acid (DHC) is a commonly used choleretic agent in pre and post operative cases of surgical obstructive jaundice in infants and children, the drug is not only expensive but also difficult to procure in our country. Frequently, the therapy has to be discontinued in absence of regular availability of the drug. In this study we have tried to find out the hepatoprotective and choleretic activities of a new Ayurvedic Indian traditional herbal drug Livzon and compare its results with those of DHC, in albino rats.

#### MATERIAL AND METHODS

Forty five Albino rats weighing 100–150 g were anaesthetised by injecting 100 mg/kg of Ketamine intraperitoneally. Laparotomy was done in each rat by a midline upper abdominal incision. Wedge biopsies were taken from right or left lobe of liver and sent for histopathological examination. The common bile duct (CBD; formed by joining of four hepatic ducts from four lobes of liver in rat — there being no gallbladder) was dissected out from where it courses along with portal vein and hepatic artery in the right edge of lesser omentum and ligated with 5–0 silk sutures. Liver was put back into peritoneal cavity after achieving complete haemostasis. The rats were divided into three groups viz:

1. Control group (15 rats): No drug was given to these rats.
2. Livzon trial group (15 rats): Livzon (400 mg/kg) was given to these rats through their water bottles.
3. Decholin group: 15 rats. Decholin (100 mg/kg) was given through water bottles.

There were no untoward effects due to drugs during this study. In each group, 5 animals were sacrificed at 3, 4 and 5 week after the ligation of the CBD. At reoperations, the liver was observed for its colour and surface texture and the bile duct was observed for degree of dilatation and quantity of bile it contained. The liver was biopsied for histopathological examination.

#### RESULTS

The gross observations in this study were as follow;

*Control group.* The ductal dilatation ranging between 3 mm and 5 mm containing 0.27 ml to 1.0 ml of bile was seen. At 3 weeks after the bile duct ligation, the histopathological changes were classical of extra hepatic biliary obstruction with marked proliferation of ductal tissue with monocytic, fibroblastic and few neutrophilic infiltration. At 4 weeks, in addition to the above findings, there were scattered vacuolation of hepatocytes. At 5 weeks, there were feathery degeneration of hepatocytes and early cirrhotic changes as demonstrated by the

interlobular bridging of ductal and fibrous tissues with the formation of nodules.

*Livzon trial group.* In this group, there was more marked ductal dilatation measuring between 3 and 12 mm and contained more amount of bile (0.5 ml–2.0 ml). Proliferation of bile ducts was also more as compared to that in the control group during the 3rd week after the duct ligation. Plenty of bile lakes were also observed. Very few hepatocytes with cytoplasmic vacuolations were seen at 5 week after the duct ligation. No feathery degeneration or cirrhotic changes were seen even after 5 week following bile duct ligation.

*Decholin group.* The dilatation of bile ducts were more than that seen in control group but less than that seen in Livzon, the histological changes were essentially same as that of control group, except that there were marked sinusoidal dilatation and scattered bile lakes. At 4 weeks, plenty of hepatocytes with cytoplasmic vacuolation were observed, which became very wide spread by 5th week of ligation of the duct.

## DISCUSSION

The changes after the ligation of CBD varied in all the three groups. In control group, after 3 weeks of ligation of bile duct, the classical features of extrahepatic cholestasis, characterised by ductal proliferation were seen but there was no evidence of cholangitis. These were comparable to the changes described by MacDonald and Pechet (1961), Moritz and Snodgrass (1972) and Johnstone and Lee (1976) in the experimental rats. After 4 weeks of ligation of the CBD, there was an increase in ductal proliferation with encroachment into parenchyma and an interlobular bridging. However, the classical changes of cirrhosis were not seen in our study. Scattered vacuolation of hepatocytic cytoplasm were seen in degenerating cells. After 5 weeks of ligation of bile duct, there were areas of feathery degeneration and the changes characteristic of early cirrhosis. However, the changes due to the advanced cirrhosis were not found.

Cirrhotic changes were also not seen by MacDonald and Pechet (1961), Moritz and Snodgrass (1972) and Johnstone and Lee (1976) even after 14 to 40 days of ligation of the CBD. It is however, reported by Franco et al. (1979) 3 to 5 weeks after the bile duct ligation in rats. No rats in this group, however, had decompensated cirrhosis with ascites, it has been reported by Cameron and Hasan (1958) following CBD ligation in rats.

In the Livzon trial group, the process of liver damage following ligation is significantly delayed and the few evidences seen are also of much milder degree. After 3 weeks of ligation the changes were; a marked ductal proliferation, pronounced monocytic (and not neutrophilic), fibroblastic and ductal cell infiltration. Plenty of bile lakes were seen in the intercellular spaces and the portal tracts. Vacuolisation of cytoplasm was observed in only a few scattered hepatocytes. Significantly no cirrhosis was demonstrable in this group.

In Decholin group, after 3 weeks of CBD ligation, there were marked dilata-

tion of sinusoids, in addition to the ductal proliferation. Scattered vacuolation of cytoplasm was observed after 4 weeks of ligation which became widespread by 5th week of ligation. However, feathery degeneration or the so called bile infarcts, as were seen in animals in the control group, were not demonstrable in this group even after 5 weeks after ligation. Small amount of bile lakes were occasionally seen in the intercellular spaces.

The surface of the liver remained smooth in all the animals except that during 5th week, the liver was granular in control group. From this study we infer that Livzon appears to be distinctly hepatoprotective and choleretic agent whereas decholin is mainly a choleretic and a mild hepatoprotective agent.

Cholagogues, choleretics and hepatoprotective drugs have been used for long clinically and experimentally by many workers like MacDonald and Pechet (1961), Desjenx et al. (1971), Moritz and Snodgrass (1972), Soloway et al. (1973) and Johnstone and Lee (1976). A cholagogue has been defined as a drug which increases biliary flow by contraction of the gall bladder and biliary tree and the choleretic as the one which increases secretion of bile by liver cells. In modern medicine, these have been used in viral hepatitis, cirrhosis, cholelithiasis, cholecystitis etc. Examples of various choleretics used are steroids, Cholestyramine, turmeric, divanillil, cyclohexamine combined with sorbitol and incesitol and sodium dehydrocholate.

DHC or Tri keto cholanoic acid is a semisynthetic bile acid. It is a colourless and bitter powder that is insoluble in water but soluble in alcohol, acetone and chloroform. It does not occur in bile under physiological conditions but has been used for many years in man to stimulate bile flow and to measure the circulation time (Wang et al. 1987). Desjenx et al. (1971) have shown that fifty percent of DHC infused is metabolised by liver into monohydroxy-diketo cholanoic acid and dihydroxy keto cholanoic acid. DHC is not excreted as a free radical in bile.

The remarkable choleretic potency of dehydrocholic acid compared to other bile salts have been explained by the fact that DHC does not form micelle in the bile, and therefore is more active osmotically than micelle-forming bile salts (Hardison 1971). As it induces bile production of a low specific gravity, it is therefore called a hydrocholeretic drug. The authors, however, feel that the dehydrocholeresis produced by DHC cannot be explained by osmosis alone because increase in the flow of bile occurs at a time when the DHC metabolites accounted for a small proportion of bile salts in bile. They feel that increment in bile flow is more likely to be because of secretory fraction. It has also been shown that hydrocholeresis is probably induced by metabolites of DHC. DHC also significantly lowers the excretion of phospholipid and cholesterol excretion in bile as had been shown by Desjenx et al. (1971) and Hardison (1971). Dehydrocholates also decrease the excretion of bilirubin and hence are not effective in attenuating jaundice. These are also contraindicated in presence of complete mechanical obstruction and hepatitis.



Livzon, a recently marketed drug, is a combination of *Phyllanthus niruri*, *Phyllanthus emblica*, *Tinaspora cordifolia*, *Terminalia chebula*, and *Terminalia belerica*. These drugs are known to have been used by millions of people in India for thousands of years. The individual components as also the combinations have been subjected to pharmacological and biochemical studies in order to elucidate the mechanism of actions of Livzon. So far the following activities of Livzon have been confirmed (Baghi, R.P., 1988; Govil, M.K., 1988, Report provided by MIS Hind Chemicals Ltd., Kanpur, India.)

*Induction of microsomal enzymes of liver.* (An increase in Synthesis of bilirubin and its conjugation). The large number of hepatic functions are carried out with the help of P-450 group of mixed oxidase microsomal enzymes. The toxic substances brought to the liver by the portal vein are subjected to metabolism, followed by conjugation with glucuronic acid to form water soluble metabolites which are then eliminated either in urine or in bile. It has been shown in laboratory animals that the enhanced liver functions under Livzon therapy lead to large percentage of metabolism of hexabarbitone and Subsequent reduction in sleeping time in rats.

*Dose related increase in the flow of bile.* Unlike other cholericics and cholagogues where the increase in bile is actually a one-time effect caused either by contraction of the gall bladder or increase in volume by osmosis, the cholericic effect of Livzon is based on the dose related increased formation and excretion of bile upto 30%, due to the improvement in the hepatocellular functions.

*Free radical scavenging activity* (absence of cellular damage). Besides the detoxication and elimination of toxic metabolites which provide hepatoprotective actions, Livzon induces the enzymes like catalases, controlling the lipid peroxidation, and acts as a free radical scavenger. This is comparable to other known agents possessing similar activity. The dose of Livzon can also be increased to many folds without encountering any side effects. This helps in preventing any damage to the liver and other organs and can safely be instituted even in the presence of hepatitis, cholestasis and other diseases of liver.

*Hepatoprotectivity.* It was evidenced by providing protection against the galactosomine toxicity and preserving the liver function tests in rats.

## CONCLUSION

Based on these findings, we conclude that Livzon is definitely a hepatoprotective and a cholericic agent in rats. It can be used for various indications like postoperative biliary atresia, neonatal hepatitis and choledochal cyst in pediatric age group. It is locally manufactured, cheaper and easily available in syrup and capsule forms. On the contrary, Decholin is mainly a cholericic drug with minimal hepatoprotective activity. As it has to be imported in India, the drug not only proves very expensive to Indian society but the regular supply also can not be ensured. We feel Livzon is a safe and effective substitute for an

hitherto, imported and expensive drug Decholin, for the use in infants with various disorders of surgical jaundice and hepatocellular damage.

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