

HEPATOBIILIARY CLEARANCE OF LABELLED MEBROFENIN IN NORMAL AND D-GALACTOSAMINE HCL-INDUCED HEPATITIS RATS AND THE PROTECTIVE EFFECT OF TURMERIC EXTRACT

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Abstract : This study was carried out to see the hepatobiliary clearance of ^{99m}Tc -Mebrofenin radiopharmaceutical in D-galactosamine induced hepatic rats. Furthermore, protective effect of turmeric extract has been studied in these hepatitis rats. Hepatitis was induced with intraperitoneal injection of D-galactosamine (400 mg/kg b. wt) in these rats. 1% turmeric extract was given along with their normal diet for 15 days.

Turmeric extract treatment significantly increased the hepatic uptake of radioactivity and accelerated the excretion of ^{99m}Tc -Mebrofenin as compared to control rats. ($P < 0.001$). In D-galactosamine administered rats, a significant delay was observed in ^{99m}Tc -Mebrofenin excretion as compared to controls. However, D-galactosamine administered rats, pretreated with turmeric extract or concurrently treated with turmeric extract showed a near normal pattern of ^{99m}Tc -Mebrofenin excretion. Hence, it can be suggested that turmeric extract may improve the liver function by detoxification.

Key words: ^{99m}Tc -Mebrofenin D-galactosamine (GalN)
turmeric extract hepatic uptake hepatic excretion

INTRODUCTION

The liver, an organ essential to life, has diverse functions for the body in substrate synthesis, storage, catabolism and detoxification. Disease states including acute and chronic hepatitis, cirrhosis and cancers can lead to changes in liver blood flow and to direct effect on the enzyme systems involved in biotransformation (1), resulting in decreased hepatic metabolism. Hence functional imaging of liver metabolism

warrants particular interest. Hepatobiliary scintigraphy using ^{99m}Tc -labeled Mebrofenin [N-(3-bromo-2, 4, 6-trimethylacetanilide)-iminodiacetic acid] is commonly used to evaluate liver function (2). It localizes within the liver and is excreted into the biliary tract during the 24 hr post injection period (3). Physiologic parameters of hepatic extraction, hepatic excretion, biliary tract patency and gallbladder contractility can be measured with ^{99m}Tc -mebrofenin (4). Our earlier work has shown that the administration of

turmeric extract provides in-vivo hepatoprotection in carbon tetrachloride induced liver damage in rats by accelerating the clearance of toxic metabolites from the liver (5). The present study with ^{99m}Tc -Mebrofenin assesses the liver function of rats with hepatitis induced by D-galactosamine, since it is known to induce acute cellular damage to the liver which resembles to that of acute viral hepatitis and effect of turmeric extract on these rats.

METHODS

Turmeric Extract

Fine powder of dry rhizomes of turmeric (*Curcuma longa*) was subjected to cold ethanolic percolation. The ethanolic extract was then evaporated to obtain a dry powder which was obtained from Saiba Industries. (Saiba Industries Pvt. Ltd., Mumbai, India) The normal diet consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined oil and 0.25% shark liver oil. In this normal diet 1% of turmeric extract was mixed for the experimental animals.

Animals

Adult male Wistar rats weighing 200–250 g, obtained from Bhabha Atomic Research Centre, (B. A. R. C.) Trombay, were used for the study. They were acclimatized to animal house condition and were fed with normal diet and water ad libitum. Animal studies were performed in compliance with B. A. R. C ethical committee guidelines.

Experimental Design

Total 150 rats were divided into five different groups, 30 in each. Group I rats

were on normal diet and served as controls. Group II were given 1% TE in their diet for 15 days. Group III rats were on normal diet and were injected with 400 mg GalN/Kg bw i.p (D-galactosamine –HC1, pH adjusted to 7.0–7.2, Sigma chemicals U.S.A) 24 hrs before the experiment. Group IV rats were given 1% TE in their diet for 15 days prior to GalN administration. TE treatment was discontinued after GalN injection and this group served as TE (Pre). Group V rats were on normal diet and were injected with GalN and then 1% TE treatment was started immediately after the injection. This group of rats served as TE (Conc). Six rats from each group were taken for every experiment so as to get% administered activity at different time intervals e.g 5, 15, 30, 60, 120 and 240 min. Five such experiments were performed.

Twenty five hours after the dose of D-galactosamine injection, the extraction and excretion pattern of ^{99m}Tc -Mebrofenin (Board of Radiation and Isotope Technology, Vashi, India) in rats from each of the five experimental groups was studied by injecting ~50 uCi (1850 kBq) ^{99m}Tc -Mebrofenin (0.2 ml) into the dorsalis penis vein of anesthetized rats. They were sacrificed at intervals of 5, 15, 30, 60, 120 and 240 min after radioactivity injection. Blood was collected from heart in heparinized syringe. Tissues excised for counting the radioactivity included liver, spleen, heart, lungs, kidneys stomach and gut consisting small and large intestine including contents. Counting was done in a broad well type scintillation counter designed for animal tissues. The carcass and site of injection were also counted to rule out anomalous injection. After counting the radioactivity from the blood sample, plasma was used for the

measurement of enzyme activity (6).

All values were expressed as mean \pm SD and biological data were analysed statistically using students t-test.

RESULTS

Biodistribution of radioactivity after i.v. injection of ^{99m}Tc -Mebrofenin is illustrated in Fig. 1 and 2. The results are expressed

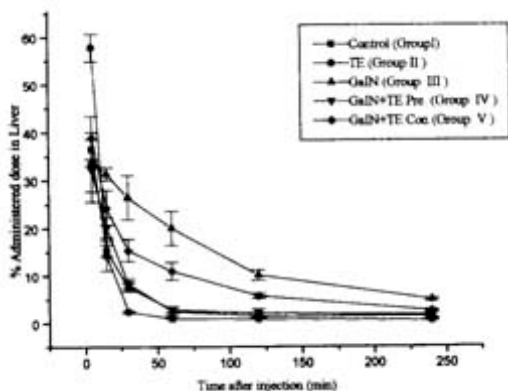


Fig. 1: Hepatobiliary clearance of ^{99m}Tc -Mebrofenin in control and experimental rats.

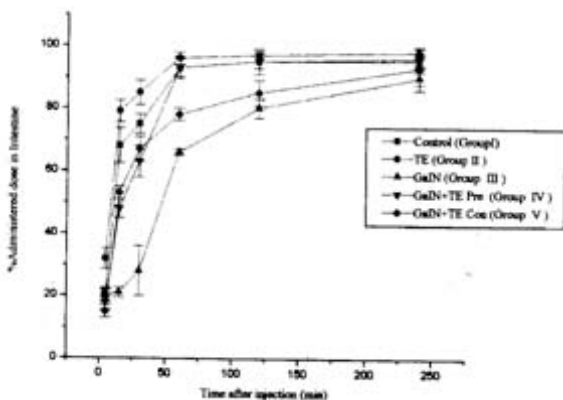


Fig. 2: ^{99m}Tc -Mebrofenin clearance from intestine in control and experimental rats.

as percentage of the injected dose in respective organs. Organs with insignificant accumulation of radioactivity were not shown.

Plasma levels of aminotransferase (AST, ALT) and alkaline phosphatase were measured in all the animals. There was a significant increase in all the liver enzyme activities after GalN administration thereby showing hepatic injury in these animals (AST: 22.7 ± 4.8 U/L to 74.9 ± 6.7 U/L; ALT: 15.5 ± 2.8 U/L to 100.3 ± 2.7 U/L; Alk. Phosphatase: 176 ± 39 U/L to 309 ± 45 U/L after GalN administration $P < 0.001$).

Tissue distribution studies at 5 min. of ^{99m}Tc -Mebrofenin injection indicate that the liver is the major site of uptake in all the groups. Rats pretreated with turmeric extract (Gr.II) showed a significantly higher percent liver uptake as compared to control rats. (Gr.I) (TE. 57.75 ± 2.9 ; Control. 36.5 ± 3.5 , $P < 0.001$), whereas rats treated with D-galactosamine (GalN. 33.8 ± 6.2) and D-galactosamine pretreated with turmeric extract (GalN. +TE Pre 32.1 ± 6.5) did not show any change in percentage liver uptake at 5 min. as compared to controls. However slight increase in percentage of liver uptake were observed in GalN rats concurrently treated with turmeric extract (GalN+TE Conc. 38.9 ± 4.5) which was not significant.

Excretion of radioactivity through kidneys at 30 mins. was significantly more in GalN-treated rats as compared to control, TE and GalN+TE (Pre) rats. [Control. 2.0 ± 0.15 , TE 0.5 ± 0.1 ; GalN. 4.75 ± 1.0 ; GalN+TE (Pre). 0.97 ± 0.15 ; GalN + TE (Conc) 3.5 ± 0.92 ; TE Vs Control $P < 0.05$; GalN Vs Control $P < 0.05$].

Fig. 1 shows the ^{99m}Tc -Mebrofenin liver

uptake and excretion pattern of control and experimental animals. A significant increase in hepatic excretion of radioactivity was observed in TE-treated rats (Gr. II). GalN administered rats showed a significant decrease in hepatic excretion of labeled compound (Gr. III). However, GalN treated rats pretreated with turmeric extract or concurrently treated with turmeric extract showed a near normal pattern of ^{99m}Tc -Mebrofenin excretion. (Gr IV and Gr V).

Fig. 2 shows excretion of radioactivity in the gut. Intestinal activity of turmeric treated rats was 85 ± 4 at 30 min. which was significantly higher as compared to controls. (Control 75 ± 3 ; $P < 0.05$). GalN treated rats showed 28 ± 8 activity in the gut which was significantly improved with turmeric treatment. (Pre as well as concurrent, GalN+TE Pre 63 ± 5 ; GalN + TE Conc. 67 ± 4 ; vs GalN; $P < 0.001$).

DISCUSSION

Turmeric (*Curcuma longa*), which has long been used in Indian cuisine as a spice, has varied physiological and pharmacological activities (7). The various constituents of turmeric were examined by Kiso et al in 1983 and have shown that turmeric causes a significant decrease in aminotransferase (AST and ALT) activities of liver cells exposed to carbon tetrachloride and D-galactosamine [8]. Recently we have reported that CC14-induced cirrhotic rats consuming the turmeric extract in diet show protective effect on liver enzymes (5).

D-galactosamine induced hepatitis is a useful model for the study of hepatic injury. D-GalN induced liver injury results from the depletion of uridine nucleotides and subsequent depression of the synthesis of macromolecules (9). This cellular damage

provokes inflammatory reactions resulting in a histological picture closely resembling acute viral hepatitis. In the present study the rats in group III, IV and V were injected with 400 mg GalN per kg body weight intraperitoneally. This dose is known to induce liver damage (10).

In this study we examined whether turmeric extract feeding influences the hepatic extraction and excretion of ^{99m}Tc -Mebrofenin in normal rats and rats treated with D-galactosamine. Indication of liver damage by D-GalN was confirmed by significantly elevated levels of plasma aminotransferases (AST and ALT) and alkaline phosphatase as compared to control rats. Though liver histological findings were not suggestive of complete reversible effect of TE, plasma aminotransferase and alkaline phosphatase levels in TE treated groups (Pre and Conc.) were significantly lower in comparison to only GalN treated group (Gr III).

Studies in humans have found that 98% of ^{99m}Tc -Mebrofenin localized within the liver and peak liver activity is usually reached within 8–15 min. The excretion phase begins after a brief hepatic transit time (3). Our present data indicate that the liver is the major site of uptake and ^{99m}Tc -Mebrofenin from the liver is more rapidly removed in turmeric treated rats than normal rats. A significant delay was observed in ^{99m}Tc -Mebrofenin excretion in D-GalN treated rats as compared to controls. However, D-GalN treated rats, pretreated with turmeric extract or concurrently treated with turmeric extract showed a near normal pattern of ^{99m}Tc -Mebrofenin excretion.

^{99m}Tc -Mebrofenin enters the hepatocytes through the dye anion receptor by a carrier mediated, nonsodium-dependent membrane transport mechanism (11). It does not

undergo biotransformation during the transit through the hepatocyte to the bile canaliculus (12). The final excretory step involves transportation across the canicular membrane. Hepatocellular disease will decrease both the uptake into hepatocyte and the excretion into the biliary tract (3). Liver uptake is mainly responsible for this rapid removal, although within the liver only paranchymal cells exert this property. The uptake by paranchymal cells is coupled to efficient biliary excretion. In our present study, only TE treated rats showed significantly higher liver uptake as compared to controls. Though control rats, GalN treated rats and GalN treated rats pre or concurrently treated with turmeric extract

showed more or less same liver uptake, their excretion pattern differs. Moreover curcumin, the active principle of turmeric is known to enhance bile acid production and secretion (7). It also enhances excretion of cholesterol in bile with concomitant reduction in bile cholesterol saturation and elevated faecal fat excretion (3). It is thus possible that higher liver uptake and increased biliary excretion in turn, may accelerate the clearance of toxic metabolites of D-GalN from the system.

The results obtained from this study show the positive role of turmeric extract as a hepatoprotector against D-galactosamine induced experimental hepatitis.

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