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A NEW RECEIPT FOR LIVER INJURY

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ABSTRACT: *A new receipe consisting of Phyllanthus fraternus Webster, Eclipta alba and curcumin was prepared. The efficacy of this preparation was tested in carbontetrachloride (CCl_4) induced fatty liver model in rats. This preparation was able to normalize the level of lipids accumulating in liver due to CCl_4 injury and it was also able to bring down elevated levels of serum bilirubin. Further, the decreased levels of serum triglycerides, pre B-lipoproteins and cholesterol in CCl_4 liver injured rats was raised after treatment with new preparation. Further, the decreased level of liver glycogen was also normalized. From these results, it can be inferred that the new receipe is able to offer protection against the liver injury caused by CCl_4 injection.*

INTRODUCTION

It is well known that in hepatic jaundice, the liver is affected. Similarly when rats are injected with CCl_4 the liver gets damaged. The herbal drugs which are mentioned to be beneficial in the treatment of viral hepatitis and toxic jaundice have been tested in CCl_4 liver injury rate model (Nazimuddin *et al*, 1981 and Khin MaMa, 1978). As a result of CCl_4 introxication, accumulation of fat in the liver and necrosis in the centribular region of the liver occur. Recknagel *et al*, 1960 have suggested that the accumulation of fat in the liver due to CCl_4 administration is due to the failure of the liver in incorporating triglycerides into lipoproteins and its transport. This defect might arise from the disorganization of endoplasmic-reticulum early after CCl_4 administration. As a consequences, the microsomal enzyme activities are found to decrease. Further, the lipid peroxidation caused by CCl_4 affects the microsonal membrane system rich in lipoproteins (Recknagel *et al* 1977). Since lipid peroxidation damages membrane lipids, the water soluble enzymes leak into plasma from the liver (Recknagel and Glende, 1973). Rao *et al* (1970) have suggested that vitamin-E, inhibitors of lipid peroxidation, free radicals scavengers and radio-protective drugs are able to protect the animals from CCl_4 induced hepatotoxicity. Our previous results (Chandra *et al*, 1985, Chandra and Sadique 1986_a, Chandra and Sadique 1986_b) have indicated that *P. niruri, E. alba* and curcumin were able to prevent the liver injury in rats subjected to CCl_4 intoxication. Therefore a recipe containing these drugs was prepared and its effect on CCl_4 induced liver injury in rats was investigated.

MATERIALS AND METHODS

Induction of fatty liver:

Male albino wistar rats weighing 150 – 200 grams purchased from private animal farm,

Bangalore were fed with rat Pellet food (Hindustan Liver Limited, Bangalore) and water *ad libitum:* The rats were injected with CCl_4 (0.5 ml / Kg.b.w.) by I.P. everyday for five days, so as to induce the liver injury (Cameron and Karunaratne, 1936).

TABLE – I

The effect of new recipe on lipid content of the liver in rats during CCl_4 induced liver injury.

No.	Group	Total lipids in mg/Kg liver tissue
1	Normal	65.4 ± 2.5^a
2	CCl_4 administered	170.5 ± 6.1
3	CCl_4 and new recipe administered	76.0 ± 2.9^a

Each value is the mean of 6 rats ± s.e.m
A= 'p' value < 0.001

Medication

Drug combination (new recipe) was prepared using shade dried leaves of *E.alba, P. niruri* and Curcumin (25:15:10). This drug suspended in 2% gum acacia was orally administered to a group consisting of 6 rats which were injected with CCl_4. Similarly, the control group received 2% gum acacia alone. The drug administration was initiated on the day of injection of CCl_4 and it was continued up to 9^{th} day. On the 10^{th} day, the animals were sacrified by decapitation, the blood was collected in test tubes and they were kept in room temperature for 30 minutes, then they were kept in refrigerator overnight, and the clear serum was prepared after centrifugation. The liver was perfused with 0.9% saline, they glycogen was separated and assayed using anthrone method (Hassid and Abraham 1957). Further, they liver lipids were extracted with a mixture of chloroform and methanol, and the chloroform fraction having lipids was evaporated to dryness. After aeration the weight was determined for total lipids (King and Wooten, 1959).

Serum analysis

Serum cholesterol (Skalar auto-analyser method), bilirubin (Wooten, 1974) and triglyceride (Soloni, 1971) were analysed. Serum was subjected to electrophoresis in cellulose acetate membranes and the percentage distribution of various lipoproteins were determined (Debalts & Lezy 1971).

RESULTS AND DISCUSSION

A drug combination containing *E. alba, P. niruri* and curcumin (25:15:10) was tested in CCl_4 induced liver injury in rats. It was found that a dose of 50mg/ 100 g.b.w was found to be effective and so this dose was

used throughout the experiment. The liver injury caused by CCl_4 is due to the accumulation of lipid in the liver (Recknagel *et al* 1960). From Table I, it can be understood that the lipid level which is elevated after CCl_4 treatment has been normalized after treatment with new recipe.

It is well known that the serum billirubin level is elevated in hepatic jaundice (varley, 1980). Similarly, there are reports to indicate that serum bilirubin level is elevated in CCl_4 poisoning (Dahiya *et al*, 1980). From Table-II, it can be noted that the elevated serum bilirubin level in CCl_4 intoxication has been reduced after treatment with new recipe, indicating the beneficial effect of the drug.

TABLE – II

The effect of new recipe on serum billirubin level during CCl_4 induced liver injury in rats.

No.	Group	Dose (mg / 100 g.b.w.)	Serum Bilirubin level mg/100 ml		
			Total	Conjugated	Un conjugated
1	Normal	-	0.28	0.28	-
2	CCl4 administered	Vehicle	0.57	0.28	0.28
3	CCl4 and new recipe administered	50	0.28	0.28	-

The values represent the average of three experiments.

TABLE – III

The effect of new recipe on serum triglyceride level during CCl_4 induced liver injury in rats.

No.	Group	Dose (mg/100 g.b.w.)	Triglyceride level (mg/100ml)
1	Normal	-	186 ± 15.2^a
2	CCl_4 administered	Vehicle	120 ± 10.5
3	CCl_4 and new recipe administered	50	160 ± 8.5^a

Each value is the mean of 6 rats ± s.e.m
a = 'p' value < 0.001

TABLE – IV

The effect of new recipe on serum cholesterol in CCl_4 induced liver injury in rats.

No.	Group	Dose (mg/100 g.b.w.)	Triglyceride level (mg/100ml)
1	Normal	-	44.52 ± 3.9^b
2	CCl_4 administered	Vehicle	42.2 ± 3.5
3	CCl_4 and new recipe administered	50	48.76 ± 4.6^a

Each value represents the mean of 6 rats ± s.e.m.
a = 'p' value < 0.005
b = 'p' value < 0.05

It is well known the immediately after CCl_4 administration, the triglyceride level in the liver is elevated (Schotz & Recknagel 1960). Further, the defect in the transport of triglycerides into plasma is the cause for accumulation of lipids in the liver during CCl_4 intoxication (Stetten & Salcedo, 1944). It has been reported that the transport of plasma fatty acids into the liver and their conversion into triglycerides occur normally even during CCl_4 intoxication. But there is an inhibition in the transport of triglyceride into plasma (Steinberg, 1963). Maximchunck and Rubinstein, (1963), have reported that within 3.5 hours after administration of CCl_4 a decrease in serum triglyceride level occurred in rats. So, these evidences are in support of the view that CCl_4 intoxication evokes a defect in the secretory mechanism of triglyceride in the liver resulting in accumulation of lipid in the liver. From Table III, it can be noted that when compared with the normal levels the serum triglycerides and cholesterol were showing lesser values in CCl_4 treated rats. However, the administration of new receipt to CCl_4 intoxicated rats has raised these values. So, it is likely that the elevations in these levels after drug administration may be due to rectification of the defective secretory mechanism of lipids associated with the CCl_4 injury. Normally triglycerides are transported into the plasma from the liver as lipoproteins. Seakins & Robinson, (1963) have reported the inhibition in the synthesis of the protein components (apoprotein) of the lipoproteins during CCl_4 intoxication. So, a reduction in the synthesis of lipoproteins will result in the lowered transport of triglycerides which will be associated with the lipoproteins. As a consequence, the plasma neutral lipids get decreased whereas liver lipid levels get increased (Seakins & Robinson, 1963). So, it is apparent that the defective secretory mechanism of triglycerides in liver poisoning is due to the inhibition in the synthesis of apoprotein of the lipoproteins. Further, it has been reported by the same workers that liver slices incubated with CCl_4 were able to incorporate C^{14}-leucine at reduced rate into plasma lipoproteins and liver proteins.

In plasma, triglycerides exist almost as lipoproteins. In the fasting rats, 65% of the total triglyceride is found associated with the very low density lipoproteins (pre β-lipoproteins) (Lombardi & Ugarize, 1965). This lipoprotein serves as the vehicle for the transport of liver-triglyceride into plasma. From Table V, it can be noticed that serum pre β-lipoprotein was only 17.2% during CCl_4 injury, whereas it showed a value of 31.4% after treatment which new drug combinations. From this result, it can be inferred that the new receipt is able to relieve the inhibition in the synthesis of apoprotein lipoprotein leading to normalization of defective triglyceride secretory mechanism.

It is well known that the administration of CCl_4 to rats leads to a decrease glycogen content of the liver (Dolak, *et al.* 1985), during the liver injury. The enzyme phosphorylase-A, which is involved in the degradation of glycogen is stimulated. But there are reports to indicate that the loss of liver glycogen is not due to stress induced catecholamine secretion (Recknagel, *et al*, 1986). Further, the activity of glycogen synthesis which is involved in the synthetase of glycogen has been reported to decrease during CCl_4 injury. So, its likely that loss of liver glycogen during CCl_4 injury may be due to either stimulation of phosphorylase-A or by stimulating glycogen synthetase, or by both the mechanisms.

From all these observations, it may be concluded that the new recipe is able to afford protection against CCl_4 induced liver INJURY. It is already established by our earlier experiments that *E.alba, P. fratuerus* hrbster and curcumin of *Curcuma longa* are endowed with the property of preventing liver damage caused by CCl_4. In addition, it has been reported that curcumin isable to prevent the lipid peroxidation (Sharma, 1976) and it also possesses anti-inflammatory frogerty (Srimal, *et al*, 1971). So it is likely that the new recipe will exert beneficial effects in human jaundice (due to viral infection or liver damage).

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