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SAFETY EVALUATION OF ICTERENE — A HERBAL MEDICINE (III. Histopathological consideration)

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ABSTRACT

In earlier studies (Zafar et al. 1993; Ahmed et al., 1993) on safety evaluation of Icetcrne, it has been observed that the drug doesn't cause any side effect as far as biochemical and hematological parameters are concerned. Present study is an extension in the same direction through histopathologic approach.

Introduction

Liver disease is the major cause of mortality and morbidity in Pakistan, and it's incidence is increasing. Viral hepatitis is one of the most important health problem, but hepatic failure due to it, or as a complication of the second common liver decease cirrhosis is a serious problem because of its considerable mortality. Long term use of salieylates, methyl dopa and konizied may give rise to chronic hepatitis (Hamadani, 1984).

The evaluation *of* cirrhosis is associated with alcohol abuse, and follows fairly predictable course. The spectrum of alcoholic liver disease includes fatty liver, central venous sclerosis, alcoholic hepatitis and cirrhosis. During early stages of fatty liver there may be little or no evidence of increased fibrous tissue with advancing disease, liver shrink to small fibrotic organ. Early in the development of postnecrotic necrosis, the liver size may be normal or enlarged. As the disease advances the liver contracts and nodules become irregular. Necrosis becomes evident (Rohhin, 1979).

Use *of* herbal drugs for providing relief from disease is as old as mankind itself. Icterene is a herbal drug used for the treatment of jaundice. Icterene is obtained from *Tornarir dioca,* (Hamdani 1984). In present report an attempt has been made to study the effect of Icterene on some vital organs such as liver and kidney in order to deter-mine the histopathological changes produced (if there is any) after administration of lam-enc. Results of test group are compared with control.

Materials and Methods

In present study, guinea pigs weighing 350-550g were used. They were divided into

5 batches, 10 guinea pigs were used as control. Guinea pigs were given food and water ad libitum.

Dosage Regimen:

The prescribed daily dose of Icterene for a patient of average weight (i.e. 70 mg) is two tablets (0.7 g each) three times a day (i.e. 4.2 g/day). Taking into account the species variation and to ensure safety evaluation for patients, experimental animals were administered five times higher dose (i.e. 100 mg/kg) three times a day through feeding tube.

Mode of Administration:

In 25 ml double distilled water Icterene (2.5 g) was dissolved and 2 ml/kg suspension was administered to animals 8 hourly. Fresh solution was prepared each day.

Duration of Treatment:

For patients 3-6 days treatment is suggested sufficient. However, to ensure complete safely evaluation of the drug, batches of animals were administered for period of 2, 4, 6, 8 and 10 weeks. The effect of the drug was studied on biochemical and hematological parameters.

For Histopathological studies animals were treated with Icterene dose i.e. for a period of 8 weeks and autopsied, after 10 weeks. Liver and kidneys were taken out. The small pieces of 2 to 3 mm were subjected to fixative Bouin's fluid for 18 to 24 hours. After the fixation, the tissue was treated with different grades of alcohol in the following order:

70 % Alcohol/hr
80 % “ “
95 % “ “
100 % “ “

Later, the tissue was treated with xylene for 1 hour in stages of 30 minutes duration. Finally the tissue is ready for embedding. The transverse sections of the tissue were prepared of 6 mu thickness. The sections of the tissue were stained in the following manner using Harriss Haematoxylin and Eosins

The section were treated with xylene for two minutes to deparaffiniscd, again treated with xylene for 2 minutes.

The sections were then subjected to dehydration with the following alcohol grades for two minutes.

1. 100% alcohol to remove xylene
2. 95% alcohol
3. 80% alcohol 2 minutes each
4. 75% alcohol
5. Distilled water

Then the sections which are dehydrated were washed to remove alcohol with distilled water. They were stained using hematoxylin stain for 11/4 or 2 minutes. Later, they were washed with tap water for 10 minutes and dehydrated with 7119, 800 alcohol. After this the sections were transferred to cosine 5'7 and again belted with 95% alcohol to wash excess stain. Then this was subjected to 1000: alcohol two changes and xylene two changes and finally covered with a cover slip using Canada Balsam.

Results

No histopathological changes *have* been observed in both organs, size of the organs also remained normal within limited sources and in view of technique employed, no changes in morphology was noted (Fig. 1-4). There was no change in histology of liver and kidney. Findings were supported by previous work (Zafar et al., 1993) that transaminase, alkaline phosphates, urea creatine and many other biochemical and hematological parameters were unaffected by Icterene. Tables show the effect of Icterene on biochemistry and hematology (Table 1-11).

Discussion

It has been revealed that Icterene shows an increase in hemoglobin concentration after 8 weeks and 10 weeks treatments. The increase in concentration of hemoglobin may be due to iron present in Icterene (8.07%) which is sufficient enough to raise the hemoglobin synthesis by incorporating iron into porphyrin. Other minerals present in Tamarix dioca are also helpful in increasing delta amino levolinic add (a precursor of porphyrin).

Results have also shown that Icterene has stimulating effect on leukocytes while slight insignificant depression of R.B.C. is seen. But after 8 weeks treatment slight increase in R.B.C. count is observed. The increased erythropoisis may be due to in-creased hemoglobin content. It is postulated that iron content of *Tanmir dioca* has some effect on glutathionc essential for the integrity of R.R.C. Thrombocyles have been found to be decreased by administration of Icterene. The reduction is statistically insignificant.

The concentration of bilirubin varied between 0.56 and 0.60 mg/dl after treatment of the drug for a period upto 10 weeks. Bilirubin is final product of haem degradation. The presence of > 15% of the total bilirubin is direct reacting form, indicates defective excretion. In contrast bilirubinemia in which > 85% of bilirubin is unconjugated, indicates an abnormality of production, hepatic uptake or conjugation.

The other type of juandice is obstructive jaundice caused either by obstruction of bile duct or by damage to liver cells, the rate of bilirubin formation is normal but bilirubin formed simply cannot pass from the blood into intestine. No remarkable difference has been observed from this study so far on bilirubin, results are statistically insignificant.

Icterene has produced slight increase in creatine and creatinine concentration in Hood of animals treated with the drug for 10 weeks. Increment is statistically insignificant. Concentration of urea in the blood of animals treated was followed upto a period of 6 weeks after treatment with the drug. The concentration of urea in test group was found to be raised. The rise is statistically insignificant.

The normal scrum enzyme levels are believed to rise from normal wear and tear, occurrence of transaminases in the plasma is almost purely the result of this action. In normal subject, the normal scrum enzymes are maintained by several mechanisms either by excretion into the bile, or urine or by inactivation.

In diseased state the raised levels are due to cell damage caused by virus or bacterial invasion which may lead to some altratio in extracellular fluid. Alkaline phosphates is an important diagnostic enzyme and increased levels are found to occur in a variety of bone and liver diseases. It is activated by magnesium ions and glutathione has inhibitory effect on this enzyme.

The alkaline phosphates is raised in post operative obstructive jaundice. Also found elevated in cholongiteis. A moderate rise in the serum alkaline phosphates may occasionally occur in infilterative disease. Raised levels are useful in indicating liver involvement (Hay et al. 1987; Powell et al. 1990).

Transaminases play a key role in intermediary metabolism as they provide a means for synthesis and degradation of amino acids in living cells, these are also involved in synthesis of urea.

The activities of transaminases in different tissues vary considerably. Elevated activities of GOT were also found in jaundiced patients. High SGOT activities in viral hepatitis and in other liver disease, including acute liver damage have been reported.

Prolonged treatment with salicylate sometimes lead to elevated transaminases. Chlorpromazine also induces jaundice accompanied by slight rise in transaminase levels (Murata et al. 1987). The present study shows insignificant differences during different time intervals, as compared to control.

The major source of energy is glucose which is always present in the surrounding extracellular fluid so that it is readily available to cells. A small amount of carbohydrate is usually stored in cell in form of glycogen, which is insoluble polyer of glucose and can be used rapidly to supply the cells energy needs. The rate of glucose transport is greatly increased by insulin. Insulin controls the rate of utilization of glucose, for the release of energy. Liver has a key role in the action of insulin in glucose homeostasis. In the basal slate glucose is continuously released by liver. The liver is also major site of glucose disposal, after a carbohydrate rich meal. Furthermore, the liver cell membrane is freely premeable to glucose

In present study the mean concentration of glucose in control group was found to be 177.4 mg/100 ml. Concentration of glucose in animals treated with the drug for 2, 4, 6, 8 and 10 weeks was found to be 164.7, 172, 176.6, 169.4 and 173.6 mg/100 ml. Creatine and many other biochemical and hematological parameters were unaffected by Icterene. Tables shows the effect of Icterene on biochemistry and hematology (Table 1-11).

Conventionally the liver is considered to be composed of regular lobules each arranged around a central vein with portal tracts at the periphery. Histologically it is evident that it is not quite true the portal tracts and hepatic tracts appear to be distributed in a slightly haphazard manner. A new concept of functional structure has been suggested. The simple unit is acinus of varying shape. The portal venule and hepatic arteriole, both send terminal branches into the acinus. They join to form a common trunk which will then contain partially oxygenated blood and this perculates through the sinusoids to several central hepatic venules. In terms of oxygen supply and nutrients 3 zones exist, Zone 1 with best supply. Zone 2 with reasonable supply, zone 3 with poorest supply. In zone 1 glycogenesis and glycogenolysis take place. This is main area of protein synthesis metabolism of protein and conjugation of drugs. Zone 3 is associated with glycogen storage lipid and pigment formation and metabolism of certain drugs and chemicals zone 2 shares functions with other zones. Zone 1, 2 and 3 form roughly concentric layers, zone 1 being the core of the complex acinus. Zone 3 is the outer layer at the periphery of the circulation.

Liver damage will interfere with secretory function and this will be reflected in blood chemistry (Scroedca et al., 1992). Some substances may be increased like plasma amino acids others may be reduced e.g. urea in case of damage (Zaflr et al. 1993); Ahmad et al.

1993). Enzymes may be related from damaged cells and appear in plasma.

Our results arc suggestive of no liver damage because levels of transaminases or phosphates are not affected by Icterene as reported by (Zafar et al. 1993). No histopathological changes are seen in liver in light of techniques employed. No changes in functions of zone 1, 2 and 3 have been reported.

Functional unit of kidney is nephron. Significant changes are not found after treatment with Icterene. Kidney is lobulated organ and its blood supply is arranged in a regular pattern which frequently determines the extent of pathological lesions. The barrier separating the blood from the lumen of the nephron consists of 3 layers, endothelium, basement membrane and epithelium. These layers form a complicated sieve controlling glomerular permeability. Wide pores exit in the endo thelium which allow passage to all chemical component of plasma including the largest protein molecules.

The epithelial cells are attached to the basement membrane by foot processes leaving pores of 300-400 °A. These pores are covered by a thin membrane which has slit 50 x 120 °A in size slightly smaller than a molecule of albumin. The main barriers to filtration are the lamina densa of the basement membrane and the slit diaphragm covering the epithelial pores.

The glomerular filtrate is virtually protein free plasma. Its rate of production is dependent upon afferent arteriolar hydrostatic pressure, plasma protein osmotic pressure, and pressure within the renal tubule. There is no change is morphology of kidney. No glomerular lesions are reported in present work. No swelling of endothelial cells or damage to basement membrane seen within limited resources.

Icterene does not alter renal chemistry. Levels of urea, creatnine are not affected by Icterene (Tahera et al. 1991; Zafar et al. 1993). It shows that Icterene doesn't affect adversely the renal chemistry. The size of the kidney also remained normal (Ahmad et al. 1993).

Icterene did not produce any morphological or histological changes on liver and kidney. It may be safely given to patient suffering from jaundice because from present and the previous reports, it may be postulated that it is safe to give Icterene since it doesn't alter biochemistry, hematology and histology of the tissues examined in present report. Long term clinical and experimental studies should be carried out to see the affect of Icterene on central nervous system and cardio vascular systems as well.

Table 1: Effect of Icterene on haemoglobin (g/dl).

Duration of Dose	Mean	±	S.D.	&
Control	13.27	±	2.08	
2 Weeks	11.4	±	2.48	8.50
4 "	10.06	±	3.44	23.00
6 "	9.43	±	1.16	27.00
8 "	12.4	±	0.94	7.60
10 "	11.23	±	2.02	15.30

Table 2: Effect of Icterene on erythrocyte count (million/cu.mm.)

Duration of Dose	Mean	±	S.D.	%
Control	5.03	±	0.94	
2 Weeks	4.68	±	0.54	6.40
4 "	4.55	±	0.05	10.20
6 "	4.01	±	0.94	20.00
8 "	3.88	±	0.90	23.00
10 "	4.00	±	1.67	20.00

Table 3: Effect of drug on leukocyte count (thousand/cu.mm.)

Duration of Dose	Mean	±	S.D.	%
Control	74.15	±	3037	
2 Weeks	7528	±	356.8	2.00
4 "	7824	±	8646	5.50
6 "	8204	±	1254.9	11.00
8 "	8216	±	9.24.4	11.00
10 "	7999	±	800	8.00

Table 4: Effect of Icterene on thrombocyte (million/cu.mm.)

Duration of Dose	%
Control	
2 Weeks	2.00
4 "	1.00
6 "	1.00
8 "	2.30
10 "	2.00

Table 5: Effect of Icterene on sgot (U/ml)

Duration of Dose	Mean ± S.D.	%
Control	110.34 ± 14.70	
2 Weeks	120.16 ± 12.60	9.00
4 "	118.4 ± 30.50	7.00
6 "	105.3 ± 16.13	4.50
8 "	121.4 ± 18.06	10.00
10 "	112.6 ± 69.70	2.20

Table 6: Effect of Icterene or SGPT (U/ml.)

Duration of Dose	Mean ± S.D.	%
Control	112 ± 25.04	
2 Weeks	120.2 ± 6.04	7.00
4 "	119 ± 32.00	6.00
6 "	128.6 ± 7.30	15.00
8 "	129.4 ± 11.20	15.00
10 "	120 ± 7.10	7.00

Table 7: Effect of Icterene on alkaline Phosphatase (U/L.)

Duration of Dose	Mean ± S.D.	%
Control	22.60 ± 13.10	
2 Weeks	27.60 ± 12.60	22.00
4 "	29.80 ± 5.50	31.00
6 "	27.30 ± 3.90	23.00
8 "	25.00 ± 8.70	13.60
10 "	25.50 ± 6.50	16.00

Table 8: Effect of Icterene on blood glucose (mg/100 ml.)

Duration of Dose	Mean ± S.D.	%
Control	177.40 ± 120.50	
2 Weeks	164.70 ± 6.40	7.30
4 "	172.00 ± 11.60	2.80
6 "	176.60 ± 12.20	-
8 "	169.40 ± 18.80	4.50
10 "	173.60 ± 13.70	2.20

Table 9: Effect of Icterene on Bilirubin (mg/dl.)

Duration of Dose	Mean	±	S.D.	%
Control	0.56	±	0.37	
2 Weeks	0.57	±	0.10	2.00
4 "	0.60	±	0.06	7.00
6 "	0.58	±	0.05	3.50
8 "	0.59	±	0.11	5.00
10 "	0.58	±	0.15	3.50

Table 10: Effect of Icterene on creatinine (mg/dl.)

Duration of Dose	Mean	±	S.D.	%
Control	0.51	±	0.19	
2 Weeks	0.55	±	0.22	8.00
4 "	0.57	±	0.05	11.80
6 "	0.55	±	0.14	8.00
8 "	0.57	±	0.17	11.0
10 "	0.58	±	0.03	13.00

Table 11: Effect of Icterene on Urea (mg/dl.)

Duration of Dose	Mean	±	S.D.	%
Control	67.70	±	16.28	
2 Weeks	71.70	±	2.14	6.00
4 "	67.60	±	2.20	0.10
6 "	72.80	±	3.40	7.40

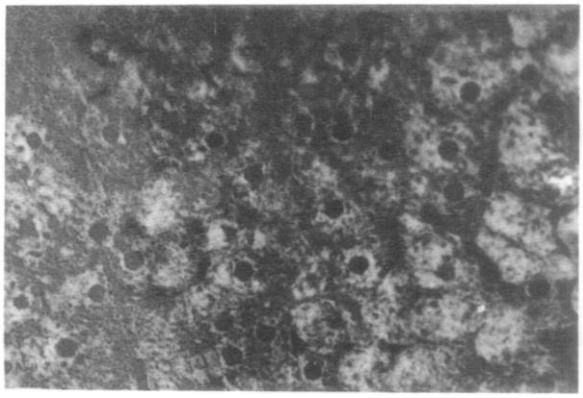

Fig. 1: T.S. of normal guinea pig liver.

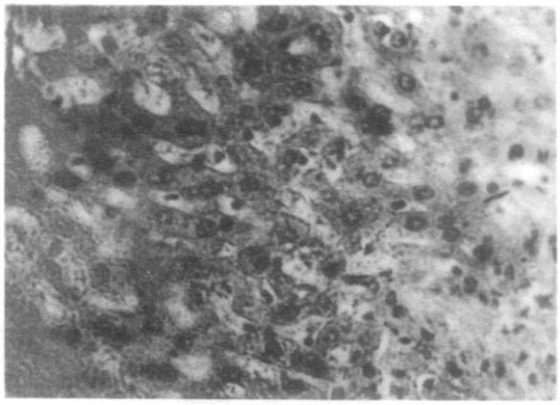

Fig. 2: T.S. of treated guinea pig liver.

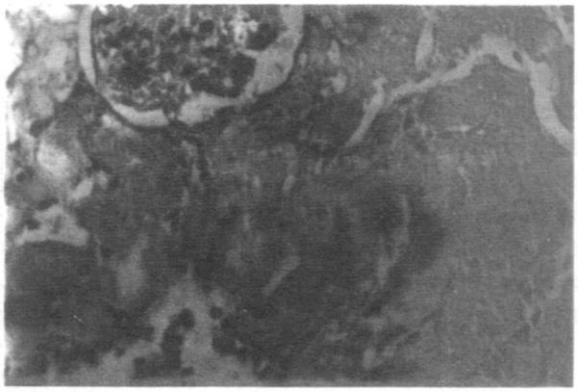

Fig. 3: T.S. of normal guinea pig kidney

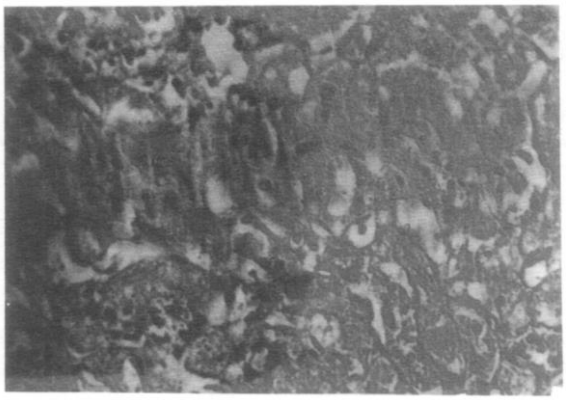

Fig. 4: T.S. of treated guinea pig kidney.

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