Efficacy of herbal coded Hepcon on drug induced hepatitis in experimental animals through histopathological and biochemical analysis

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Abstract: Drug-induced liver injury is the leading cause for more than 50 percent of cases of acute liver failure. This study was conducted on herbo-mineral formulation "Hepcon" to evaluate its hepatoprotective effects in drug induced hepatitis in experimental animals. The liver injury was introduced with over dosage of non steroidal anti-inflammatory drugs (NSAIDs) and carbon tetrachloride (CCl_4). The herbo-mineral formulations "Hepcon" consist of *Zingiber officinale*, *Piprum nigrum*, *Ammonium chloride* and *Arsenic trioxide* (Hartal warqi). The aqueous extraction was administered to experimental animals. Thereafter their LFTs, IgG, and tissue pathology was studied. It was observed on the basis of biochemical and histopathological analysis that animals which were subjected to Hepcon became normal in 60 days whereas those as control group did not showed improvements and most of them died. It was concluded that the efficacy of Hepcon to treat liver injury caused by CCl_4 and NSAIDs is very effective, and no side effects were noticed.

Keywords: Drug induced hepatitis, efficacy, Hepcon, experimental animals.

INTRODUCTION

Hepatitis is the swelling and inflammation of the liver characterized by the presence of inflammatory cells in the tissue. Hepatitis may also occur with limited or no symptoms. Hepatitis is acute when it lasts less than six months and chronic when it persist longer (Goldstein, 2000). Drug-induced hepatitis, viral hepatitis A, B, C, D, and E, alcoholic hepatitis, autoimmune hepatitis, obstructive hepatitis, alpha 1-antitrypsin deficiency and ischemic hepatitis are the most common types of hepatitis (Koziel and Peters, 2007). William Bernal and co-workers have put forth the genesis that acute liver failure is a rare disorder with high mortality and resource cost. In the developing countries, viral causes dominated by infection with hepatitis C are recognized as a common cause in many countries. In the developed countries, the incidence of virally induced disease has declined significantly in recent years, with most cases now arising from drug-induced liver damage, often from paracetamol (Bernal and Auzinger, 2010; Tajiri *et al*., 2008).

Drug-induced hepatic injury is the most common reason given for withdrawal purchase an approved drug, and representing also for more than 50 percent of cases of acute liver failure in different countries around the world. The recent endeavor has been directed toward a better understanding of these facts in order to improve results and contain drug induced liver injury (Holt *et al.*, 2009; Peker *et al*., 2006).

Drug-induced liver injury is the leading cause for more than 50 percent of cases of acute liver failure. More than 75 percent of cases of idiosyncratic drug reactions have given way to option for liver transplantation that has lead to death in some cases. In order to improve the drug not to result to drug-induced liver damage the pathogenesis of drug-induced liver damage, common adverse drug reactions are to be understood in a better experimental and clinical findings and this should be monitored exclusively to check the types of malaise (Lee, 2003).

Drug-induced hepatotoxicity involved in half of cases of acute liver failure, with paracetamol as the main factor responsible for the violation. The liver damage caused by the drug in 2.3% of patients hospitalized for jaundice. However, drugs used in drug-induced toxicity could not be figured out because of the difficulty in diagnosis and the low frequency of furnishing the data on pharmacovigilance. Therefore drug-induced toxicity represents a clinical challenge due to the large number of reported hepatotoxic drugs in use, the wide range of liver damage because of absence of clinical findings and the diagnosis on the effect on liver. Therefore the assessment of drug-induced liver injury should be the first priority while developing dosage form design (Kaplowitz, 2011). Hepcon has been developed as a Unani/traditional formulation to reveal hepatoprotective effects in hepatic inflammatory and toxic conditions.

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MATERIAL AND METHODS

Preparation of Test drug (Hepcon)

The coded formulation Hepcon consists of *Piper nigrum* (Black pepper), *Zingiber officinale* (Ginger), *Ammonium chloride* (Naushader), and *Arsenic trioxide* (Hartal warqi). All the drugs were purchased from local market and authenticated by Prof. Dr. Usman Ghani Khan, Department of Pharmacognosy, Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan. Dried ginger and black pepper was dried in oven at 60^{o}C for 5 minutes in order to remove all the moisture from it. Then both herbs were crushed in wrist motor for 15 minutes each and poured in an air tight vessel. Six flasks were washed with detergent with fresh water and dried and heated at 100^{o}C in the electric oven for 10 minutes. Then these were taken out and kept at the room temperature. After 5 minutes when the flasks were cooled down each flask was filled with 200 ml of distilled water. Then 20 grams of crushed ginger powder and 20 grams of crushed black pepper powder were added to the each set of 3 flasks. In the same way 20 grams of crushed black pepper powder per flask was added to the 3 flasks. The mouth of these 6 flasks were covered with cotton and aluminum foil in order to make them air tight. These six flasks were fixed on the Orbital shaker at 60 cycles per second for 45 hours. Flasks were removed from the shaker. Water in the flasks was changed according to the ingredient color. The flasks were then put for sonification for 15 minutes. After the sonification they were carried to the hood. In hood the solution plus plant material was filtered with filter paper in an air tight container. Two containers one of ginger extract and one of pepper extract were tightly caped and both the container was kept at 4^{o}C in a chiller Hitachi. The remaining two ingredients *Ammonium chloride* and *Arsenic trioxide* were grinded in a grinder up to 74 micron size for 15 minutes and were packed in an air tight container. Now according to the weight proportion two plant extracts one mineral salt and one heavy metal were mixed in a glass container in the hood in order to administer to the animals. 60 BD Falcon tubes of 50 ml size were filled with the medicine according to the dose of the animals and were kept in the chiller at 4^{o}C. The four containers were kept intact and also kept at 4^{o}C in order to keep protected from degradation by high temperature.

Selection and preparation of animals

36 New Zealand Male Albino rabbits were used, weighing 2.0 ± 0.2 kg. 6 rabbits were used in the experiment kept normal in the very same room (blanks). 30 rabbits were divided into 2 main groups depending on the type of chemical agents administered.

Group 1 which was administered with (NSAID) was composed of 15 animals further divided into 2 sub groups A and B. Animals labeled A1 to A9 and B1 to B6.

Group 2 which was administered with CCL_4 was also composed of 15 animals and further divided into 2 sub groups D and C. Animals labeled D1 to D9 and C1 to C6.

All the (36) rabbits were kept in a specific (the purpose built) room in the animal house of the Department of Veterinary sciences University of Agriculture Faisalabad, under the controlled conditions of ventilation, air, light and temperature. Prior to the experiment the animals were tested for LFT, CBC, and ESR etc. Reports were received on the next day and all the animals were found absolutely normal serologically (table 1).

Table 1: Test report values before induction of hepatotoxicity

Test	Average values	Normal value
Protein total	7.0	6-8 gm%
Albumin	4.0	3.5-5.0 mg%
Bilirubin Total	0.25	0.1-1 mg%
Bilirubin Direct	0.2	0.1-0.4 mg%
Bilirubin Indirect	0.3	0.1-0.7 mg%
Alkaline Phosphatase	60	20-140 IU/L
SGPT (ALT)	45	7-56 U/L
SGOT (AST)	30	5-40 U/L
IgG	12.6	0-20 GPL

Carbon tetrachloride induced liver injury

The 15 rabbits were subjected to hepatic injury by inducing 99.5% carbon tetrachloride which was mixed with paraffin oil (1:4 vol/vol) and were administered orally for 10 days. Then carbon tetrachloride induced control group was left free to cure by itself and carbon tetrachloride Hepcon group was administered with the coded drug Hepcon.

Non steroidal anti-inflammatory drugs induced liver injury

The 15 rabbits were subjected to liver injury by orally administrating Non steroidal anti-inflammatory drugs (NSAIDs) such as Paracetamol and Ibuprofen. The dosage of single animal was 200mg Ibuprofen and 250mg of Paracetamol mixed with 10ml of water were administered orally for 10 days 225mg/kg. The NSAID control group was left free to cure by itself and NSAID Hepcon group was administered with coded drug Hepcon.

From the next day all the 15 healthy animals of group 1 received doses of the two NSAIDs in order to induce hepatitis or liver injury according to the following medicine chart.

Ibuprofen 200mg tablet, 1 tablet for 1 animal; weight of single tablet = 0.45 gram, weight of 15 tablets = 0.45×15 =6.75 gram,

Table 2: NSAIDs control group average values of recovery

Test	13-8-11	26-8-11	10-9-11	25-9-11	Normal value
Protein total	6.06	6.7	6	6.2	6-8 gm%
Albumin	3.68	12.6	4	4.4	3.5-5.0 mg%
Bilirubin total	0.5	0.3	0.5	0.4	0.1-1 mg%
Bilirubin direct	0.2	0.2	0.2	0.2	0.1-0.4 mg%
Bilirubin indirect	0.2	0.1	0.2	0.2	0.1-0.7 mg%
Alkaline Phosphatase	290	269	150	98	20-140 IU/L
SGPT(ALT)	90	85	78	62	7-56 U/L
SGOT(AST)	87	72	58	38	5-40 U/L
IgG	12.6	9.6	8.6	17	0-20 GPL

Table 3: NSAIDs Hepcon group average values of recovery

Test	13-8-11	26-8-11	10-9-11	25-9-11	Normal values
Proteins total	6.06	7	5.8	6	6-8 gm%
Albumin	3.68	4	4	4.4	3.5-5.0 mg%
Bilirubin total	0.5	0.3	0.5	0.6	0.1-1 mg%
Bilirubin direct	0.2	0.2	0.2	0.2	0.1-0.4 mg%
Bilirubin indirect	0.2	0.1	0.2	0.2	0.1-0.7 mg%
Alkaline Phosphatase	139	137	80	61	20-140 IU/L
SGPT(ALT)	85	72	60.5	50	7-56 U/L
SGOT(AST)	37.6	34	16.5	17	5-40 U/L
IgG	12.6	10.5	17	16.6	0-20 GPL

Table 4: CCl_4 control group average values of recovery

Test	13-8-11	26-8-11	10-9-11	25-9-11	Normal values
Proteins total	5.88	6.5	6	5.9	6-8 gm%
Albumin	3.4	3.5	4.1	4.45	3.5-5.0 mg%
Bilirubin total	0.8	0.5	0.4	0.4	0.1-1 mg%
Bilirubin direct	0.5	0.2	0.2	0.2	0.1-0.4 mg%
Bilirubin indirect	0.5	0.2	0.2	0.2	0.1-0.7 mg%
Alkaline Phosphatase	228.6	200	150	100	20-140 IU/L
SGPT (ALT)	663.2	350	250	198	7-56 U/L
SGOT (AST)	374.2	300	235	169	5-40 U/l
IgG	41.4	15.6	9	15	0- 20 GPL

Table 5: CCl_4 Hepcon group average values of recovery

Test	13-8-11	26-8-11	10-9-11	25-9-11	Normal values
Proteins total	5.88	7.3	6.2	6.4	6-8 gm%
Albumin	3.4	4	4.2	4.6	3.5-5.0 mg%
Bilirubin total	0.8	0.8	0.4	0.4	0.1-1 mg%
Bilirubin direct	0.5	0.4	0.2	0.2	0.1-0.4 mg%
Bilirubin indirect	0.3	0.4	0.2	0.2	0.1-0.7 mg%
Alkaline Phosphatase	228.6	117	100	72	20-140 IU/L
SGPT (ALT)	542	83	75.5	50	7-56 U/L
SGOT (AST)	247	125	55	26	5-40 U/l
IgG	41.4	22	14	15.6	0-20 GPL

Paracetamol 500mg tablet, Half tablet for 1 animal, weight of single tablet = 0.57 gram, weight of 7 tablets =0.57×7 = 3.99 gram, Whole weight of dry medicine is = 6.75+3.99 = 10.74., Water added as solvent = 150ml, Drug +water solution= 10.74gram + 150ml, Dose of the single animal = 10ml, In each 10ml dose of single animal there is 200mg of Ibuprofen and 250mg of Paracetamol. The medicine is given orally with the help of stomach tube.

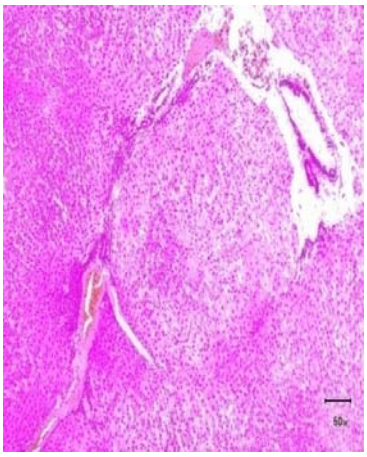

Fig. 1 Normal liver tissue from normal group

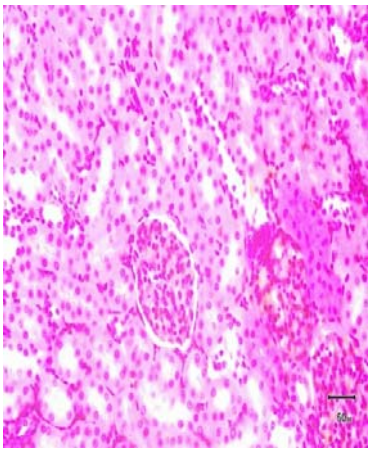

Fig. 2 Normal kidney tissue from normal group.

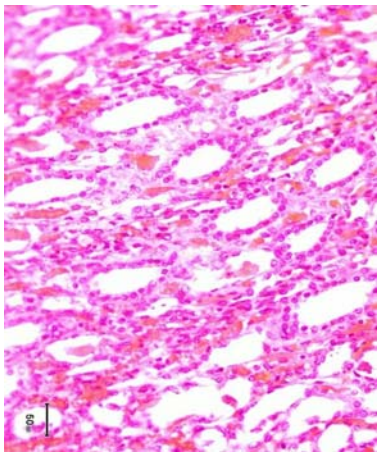

Fig. 3 Kidney with vascular congestion after induction of hepatitis.

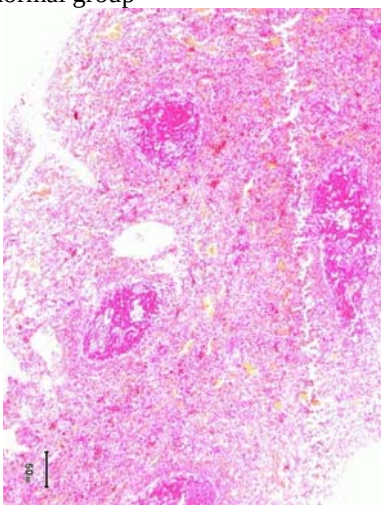

Fig. 4 Reactive lymph node after

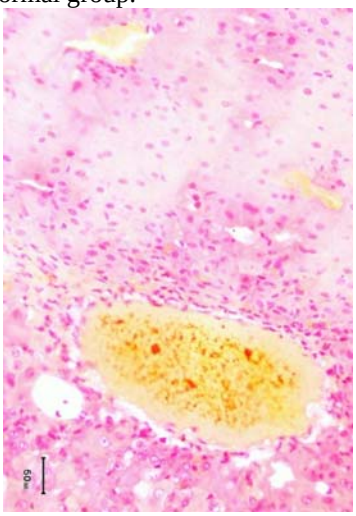

Fig. 5 Liver necrosis in NSAIDs

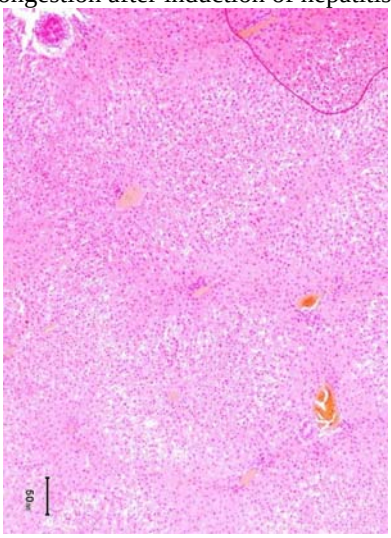

Fig. 6 Normal liver parenchyma after

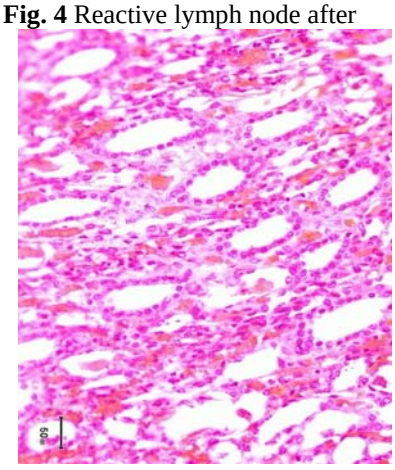

Fig. 7 CCL_4 control group kidney tissues with vascular congestion.

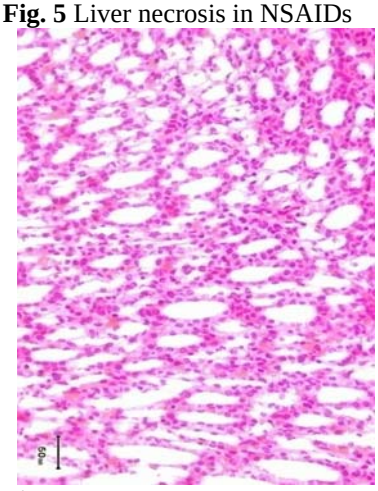

Fig. 8 CCL_4 Hepcon group normal kidney after treatment with Hepcon.

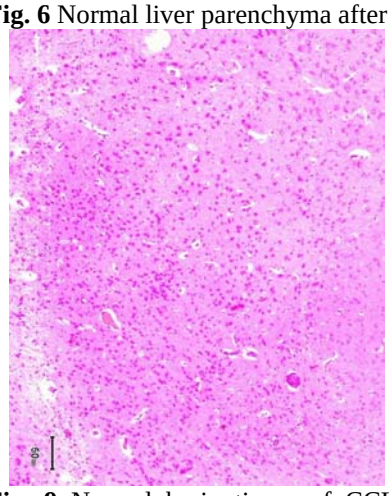

Fig. 9 Normal brain tissue of CCL_4 Hepcon group after treatment with Hepcon.

On the very same day group 2 begins to receive CCl_4 dose in order to induce hepatitis or liver injury according to the following chart. Dose of single animal of CCl_4 = 1ml per kg of body weight, Total weight of the 15 animals = 30kg, total amount of CCl_4 according to the weight of animals = 30ml, Total amount of paraffin oil added to organic

solvent = 45ml, Total amount of the mixture = 75ml, Dose of single animal=5ml. CCl_4 was purchased from the market. M=153.82g/mol, 1Liter=1.59kg. The organic solvent plus paraffin oil was given orally with the help of stomach tube.

Both groups received relevant materials for hepatitis induction in doses once a day early in the morning before their feed for 10 days regularly. On eleventh day the blood samples were collected from both groups in order to check the status of their liver health. Blood reports indicate raised values of ALT, AST and other liver enzymes four times to the normal value. Two animals from group 1 (A9 and A8) were killed in order to check the harmful effects of NSAIDs on their liver, kidney, spleen, or brain tissues. Two animals from group 2 (D9 and D8) were killed in order to check the harmful effects of carbon tetrachloride on their liver, kidney, spleen and brain tissues.

With the conformation of various blood and serum values indicating liver tissue damage and pathologically inflamed condition of liver, the animals of both groups were started to be treated with test drug Hepcon in order to check its curing effect on the drug induced or organic solvent induced liver injury. 7 animals of group 1(A1 to A7) were administered Hepcon and other 6 (B1 to B6) were kept control to cure on their own (control) so that by comparing these two groups, we can evaluate the effect of Hepcon on drug induced liver injury. Similarly 7 animals of group 2 (D1 to D7) were administered Hepcon and other 6 (C1 to C6) were kept control to cure on their own so that by comparing these two groups we can evaluate the effect of Hepcon against carbon tetra chloride induced liver injury.

Hepcon is administered to the 14 animals of two groups according to the following chart. Dose of Hepcon = 2mg per kg body weight, Dose of single animal = 4 mg, Dose of 14 animals = 4×14 = 56 mg, Total amount of tap water added = 70 ml, Mixture containing 70ml water and 56 mg medicine. 5 ml of Hepcon was administered orally with the help of stomach tube to 14 animals of both groups. Now along with Barseem dry grains, onions, potato, apple and almonds were added to the diet of all 26 rabbits of both groups. Hepcon was administered to animals for 15 days regularly at 7 am in the morning. After 15 days blood samples were taken in order to check their health status of all the Hepcon or control group animals. The blood is taken from their jugular vein centrifuged and serum sent to the laboratory for the diagnostic tests. Blood samples were taken three more times with 15 days following same pattern. After the fourth blood samples which were collected on the 60^{th} day of the start of the trial all the Hepcon treated animals were declared normal in the light of pathological reports. Administration of Hepcon is stopped. Two animals from each group (A, B, C, D) were selected to be killed for histopathological examination of their liver, kidney, spleen and brain tissues.

Two animals from Normal group were killed in order to compare with eight animals from group 1 and group 2 so that the histological changes between the normal group, control group and Hepcon group can be compared to check the positive or negative effects of Hepcon on animal tissues.

Preparation of stained slides for histological examination

Slides were prepared for histologic evaluation and stained by the procedure Erlich's Haematoxylin, 30 mins; Washed in tap water, differentiated in 1% acid alcohol dip; Blue" in tap water or Scott's Tap water 5-10 min; 5% aqueous yellowish 10 min; Differentiated in tap water; Dehydrated in absolute alcohol 2 min, repeat; Cleared in Xylol 3 min; Mounted in D.P.X, Slides were ready for examination. Tissues were added to the slides during sectioning process after cutting them with the help of microtome and these slides were dried on a slide bench.

Procedure of pathological tests performed

Protein totals were determined by Burette method; Albumin by BCG Dye Binding method; Bilirubin Total by the Jendrassik and Grof method; Bilirubin Direct by Jendrassik and Grof method; Bilirubin Indirect by Jendrassik and Grof method; Alkaline Phosphate by P-nitrophenol method; Alanine amino transferase (ALT) by Colorimetric End-point method; Aspartate amino transferase (AST) by Colorimetric End-point method and Immunoglobulin G (IgG) by Radin Elisa kit for IgG method.

RESULTS

Control group

Following are the average values of the control (NSAIDs) group in which animals were allowed to cure at their own without any medical support. Out of six animals two died during this two month course, the first after 16 days and the second after 27 days. On their autopsy lesions were found on their intestines, liver was enlarged and dark bluish in color. On cutting their skull lesions were also found in their brain tissue. They showed poor prognosis in 60 days as compared to the animals treated with Hepcon. Their diet and drinking habits were again very much altered as compared to Hepcon treated group. They showed less interest in food and consumed less amount of water as compared to Hepcon treated group. Body reflexes were also slow. Color of their urine was very dark brownish and they seemed to feel un-easy during micturation. After the induction of hepatitis there was an increase in temperature of $1^{o}C$ above the normal which slows down with the passage of time but still remains at the highest normal value. Their weight was reduced after the induction of hepatitis which they could not recover.

Six rabbits which were kept normal (Blank) in the experiment remain alive till the end of study because the rabbits involved in the experiment remain sufficient to carry out all experimental procedures. The Hepcon group which was treated with herbo mineral coded drug Hepcon after the induction of hepatitis showed excellent response to inflammatory condition. From the very first day of Hepcon administration their physical and chemical conditions started to improve. They also lost weight and food interest when hepatitis was induced. Body reflexes were also slow and dark brown urine after micturation with pain but, following treatment with Hepcon with the passage of time they improved and showed interest in food and water and also gained the weight. Color of their urine became normal. Their temperature also became normal after two weeks and there was no further death of any rabbit from this group.

Following are the results of carbon tetrachloride (CCl_4) control group. When carbon tetra chloride was administered to the animals along with paraffin oil through the stomach tube they seem very uncomfortable, restless and disturbed. They abruptly increase temperature, loss of appetite, reduced water intake, dark black urine, laziness and altered reflexes. After ingestion of CCl_4 they remained restless for an hour after which they became normalized slowly. They reduced their body weight up to 200g on average. Their ear veins became prominent. With the passage of time they overcome their symptoms of toxicity developed slowly interest towards food and water and gained their body weight but as compared to Hepcon treated group these advancements were very slow and there was a prominent difference between these two groups.

The animals of CCl_4 Hepcon group also seemed very much tense when they were subjected to hepatitis induction period. Sudden weight loss, loss of appetite, reduced water intake, elevated body temperature, dark colored urination, restlessness, and altered reflexes. But as compared to CCl_4 control group when Hepcon was administered to them their severe symptoms disappeared within few days. Temperature became normal, their appetite and water intake increased and they gained their respective body weight. Color of urine became normal.

DISCUSSION

Drug-induced hepatotoxicity has been dealt with in a communication by William M. Lee. He stated that Drug-induced liver injury is a growing problem along with complication of drugs prescribed, because the liver is for the metabolic conversion of drugs and foreign material. Although drugs are supposed to metabolize without damage, the liver damage has been constantly observed an adverse event. Drugs and their metabolites may cause liver damage in a single dose fashion. Most drug material is a toxic byproduct only in rare individuals. Injury to hepatocytes results either directly by the disruption of intracellular function or membrane integrity or indirectly by immune-mediated membrane damage. Genetic alterations in enzymes, harmful metabolite competition in drugs, and depletion of substrates are required to detoxify the metabolite in the liver so that it can be prevented (Schapira and Basan, 1986).

Drug-induced hepatotoxicity has resulted in withdrawal of many drugs that produced severe liver damage, a potential risk that was not recognized in the pre-approval of clinical trials. The recent view of research undertaken by Dr. Tai-nin Chau on drug-induced liver injury describes that the function of liver is to metabolize (foreign) substances and also transport it to the site of absorption and systemic circulation. More than 1000 drugs are utilized with idiosyncratic hepatotoxocoty and drug-induced liver injury. This has created an awareness as well as point for removing viable drugs from the market (Chau, 2008; Khoo, 2003).

The predominant clinical picture is acute hepatitis or cholestasis with clinical pathological parameters of acute or chronic liver disease and appears as a core/main committing outcome. The pathogenesis of drug-induced liver disease involves the participation of the parent drug or metabolite(s) that either directly affects the biochemistry of cells or to elicit an immune response. Each hepatotoxin is associated with a distinctive signature on the pattern of loss and latency. Unpredictable, low frequency, reactions occur in the background by a higher rate of mild asymptomatic liver injury and these are difficult to detect by monitoring serum alanine aminotransferase levels. The investigation on development in toxicogenomics and proteomics could improve the determination of risk factors and exploration of idiosyncratic hepatotoxicity (Horng-Yuan Lou *et al*., 2011; Makol, 2011).

CONCLUSION

It is concluded that Hepcon is effective to treat liver injury caused by CCl_4 chloride and NSAIDs and no side effects were noticed. All rabbits subjected to liver injury and then treated with coded formulation Hepcon exhibited normal reports and that liver biopsy examined was also normal. In addition the kidney and brain tissues which is usually affected by NSAIDs and CCl_4 also displayed that no effects were seen in both the tissues in Hepcon treated animals. Consequently it is noted that Hepcon has the potential to cure liver injury caused by either drugs or organic solvents like alcohol.

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