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Hepato-Protective Effect of *Karisalai Karpa Chooranam* against Paracetamol Induced Hepato Toxicity in Zebra fish (Daniorerio) Model

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Abstract:
Background: *The liver may be considered as the most important organ in drug toxicity because it is functionally interposed between the site of absorption and the systemic circulation and is a major site of metabolism and elimination of foreign substances; these features render it a preferred target for drug toxicity. Drug-induced liver injury (DILI) therefore possess a major clinical problem. DILI is initiated by direct hepatotoxic effects of a drug. Paracetamol, a widely used analgesic and antipyretic drug, which causes acute liver damage in higher doses in both animals and in humans. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. Exposure of Paracetamol in Zebrafish causes 75% reduction in liver antioxidant enzyme level within 24 hours post exposure.* ***Aim****: The present study is aimed to evaluate the hepato protective effect of Siddha preparation Karisalai Karpa Chooranam (KKC) in Zebrafish model.* ***Materials and methods****: In this study, the liver injury was induced by Paracetamol and the test drug KKC has been administered at the dose of 250 and 500 mg/liter for seven days. After one-week exposure period, the liver of Zebrafish was dissected and histopathological studies were performed.* ***Result****: The result of this present investigation indicates that Paracetamol treated groups show severe liver degeneration whereas treatment with test drug KKC at both the dose levels significantly attenuated the Paracetamol induced damages.* ***Conclusion:*** *This study concluded that the drug KKC possesses promising hepato protective activity in dose dependent manners and restores the basic liver architecture by means of its rejuvenating potential against Paracetamol induced toxicity in Zebra fish model.*

Key Words: *Siddha medicine; Karisalai Karpa Chooranam; Hepato – Protective activity.*

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I. Introduction

Liver is the most important organ of living system and performs vital functions in regulations of physiological processes in the body. It is involved in metabolism, secretion and storage. Liver diseases are mainly caused due to excess consumption of alcohol, toxic chemicals, infectious diseases and autoimmune disorders may also be the major cause. Diseases like jaundice, liver cirrhosis, and fatty liver disease are commonly occurring worldwide. [1]

Liver diseases have become one of the major causes of morbidity and mortality all over the world. From among, drug induced liver injury (DILI) is one of the most common causative factor that possess a major clinical and regulatory challenge [2]. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure [3].

In recent years there has been tremendous advancement in the field of science and hematology; however liver problems are on high rise, the two major liver diseases which has caused high death rate annually account for jaundice and hepatitis [4]. Hepatotoxic chemicals damage liver cells by inducing lipid peroxidation and other oxidative damages [5]. More than 900 drugs have been found to cause liver diseases and have been stopped from implications [6].

Drug induced liver injury has become a major challenge. Various models have been identified for predicting the toxicity, Paracetamol, a widely used analgesic and antipyretic drug, which causes acute liver damage in high doses in both animals and in humans. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. A hepatotoxic metabolite, NAPQI (N-acetyl-p-benzoquinone imine) that is produced by cytochrome P450 of liver enzymes, remain inactivated at therapeutic doses by Liver GSH [7, 8].at higher doses,

these metabolite gets activated due to decrease in level of GSH, which accounts for the dysfunction of liver, causing mitochondrial DNA damage, oxidative stress and apoptosis of immune cells [9-11].

In spite of advancement in the recent trends in drug development, presently only a few hepatoprotective drugs are available for the treatment of liver disorders [12]. Various natural remedies from traditional medicinal plants, ie in Siddha have been found to be effective against liver damages and has been recommended for the treatment of liver diseases in the Indian system of medicines [13]. The relative accessibility, low prices, local availability and acceptance by the local communities have made heavy reliability on plant medicine. Since the ancient time, plant medicine is an important part of healthcare system. In Siddha system , there are many herbal formulations available which helps in preventing liver damage [14].

In the present study an attempt has been made to screen the hepatoprotective effect of *Karisalai Karpa Chooranam* on paracetomol induced liver injury in Zeebra Fish.

II. Materials and Methods

2.1 Karisalai Karpa Chooranam [15]:

The test drug *Karisalai Karpa Chooranam* has been purchased from SKM Siddha and Ayurvedha Pharmacy, Erode, Tamilnadu. It is a poly herbal Siddha preparation prepared from seven herbs (Table: 1) which is indicated for *Paandu* (Anaemia), *Kaamalai* (Jaundice), *Kalleral veekkam* (Hepatomegaly), *Sobai* (Generalized oedema), Skin diseases and helps to enhance the immune system. It is a powerful rejuvenating medicine in Siddha system and used as a *Kaayakalpam*.

2.2 Experimental Animals :

Adult Zeebra Fish (Daniorerio) were purchased from the local supplier and were maintained in a laboratory condition 28 °C $\pm$ 1°C and a period of 14:10 h light/dark cycle photo period. All fishes' were acclimatized to lab condition four weeks prior to the start of experimentation. Animals were divided in to four groups of 10 fish each.

Grouping

Group I : Control
Group II : Paracetamol 5mM (755.8mg) per liter concentration
Group III : Paracetamol 5mM + *Karisalai Karpa Chooranam* 250 mg/liter
Group IV : Paracetamol 5mM + *Karisalai Karpa Chooranam* 500 mg/liter

2.2 Treatment

Animal belongs to Group I treated as Control and Group II treated with Paracetamol at the concentration of 5mM (755.8mg) per liter concentration for the period of seven days. Animal belongs to Group III treated with test drug *Karisalai Karpam Chooranam* (KKC) at the concentration of 250 mg/liter and Group IV treated with test drug *Karisalai Karpam Chooranam* (KKC) at the concentration of 500 mg/liter along with Paracetamol 5mM for the period of seven days.

2.4 Histopathology :

After one-week exposure period, the livers of Zebrafish were dissected and fixed in 10% formalin for 24h. Subsequently, the fixed liver tissues were dehydrated in gradient ethanol, hyalinized in xylene, and embedded in paraffin wax at 56 °C. Then, the paraffin blocks were sectioned at 5-μm thickness. The sections were collected on glass slides and stained with hematoxylin and eosin (H&E) using an H&E Staining Kit. Histologic lesions were observed using an optical microscope equipped with a digital camera.

III. Results and Discussion

The results obtained from the present investigation indicates that Paracetamol treated groups shows severe liver degeneration whereas treatment with test drug *Karisalai Karpa Chooranam* at both the dose levels significantly attenuated the Paracetamol induced damage in group III and IV. Hence from the study it was concluded that the drug *Karisalai Karpa Chooranam* possesses promising hepato protective activity in dose dependent manners and restores the basic liver architecture by means of its rejuvenating potential against Paracetamol induced toxicity in Zebra fish model.

In the present study, the histopathological studies were carried out to check the hepatoprotective effect of *Karisalai Karpa Chooranam* on Paracetamol induced liver damage. The H&E staining of the liver tissue of Paracetamol and post treated group as shown in Figure: 1. shows the control group with normal liver morphology with nomal liver cells, Figure: 2. Paracetamol treated group which shows the degeneration of vacuoles in the cell, and necrotic aggregation of cells, Figure: 3&4. shows the post treatment with *Karisalai Karpa Chooranam* indicating the regeneration of vacuoles, with no necrotic zone, and normal hepatocytes.

The Paracetamol liver injury resulting in degeneration of cells may be due to the accumulation of non-adipose tissue which results in cell necrosis and cell death and Treatment with *Karisalai Karpa Chooranam* resulted in regeneration of vacuoles and liver cells indicating that the *Karisalai Karpa Chooranam* decreases the oxidative hepatic injuries such as necrotic aggregations due to Paracetamol.

IV. Conclusion

This study concluded that the test drug *Karisalai Karpa Chooranam* possess hepatoprotective effect against Paracetamol induced liver damage in Zebra fish. This effect may due to the presence of natural phytochemicals and anti oxidants in the test formulation. Further clinical studies to be conducted to confirm its therapeutic effect on Liver diseases.

Table: 1. Ingredients of *Karisalai Karpa Chooranam*

S.No	Siddha Name	Botanical Name	Quantity
1	*Vellai Karisalai*	Eclipta prostrata	10 gm
2	*Manjal Karisalai*	Wedelia chinensis	10 gm
3	*Kuppaimeni*	Acalypha indica	10 gm
4	*Seruppadai*	Coldenia Procumbens	10 gm
5	*Vallarai*	Centella asiatica	10 gm
6	*Neeli*	Indigofera tinctoria	10 gm
7	*Kottai Karanthai*	Sphaeranthus indicus	10 gm

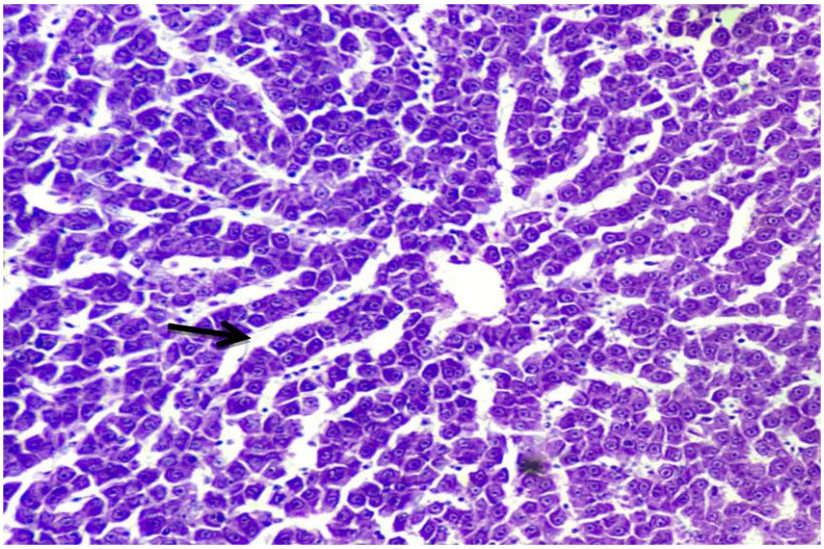

Figure: 1. Control Group: Regular sinusoidal space with perfectly aligned hepatocytes

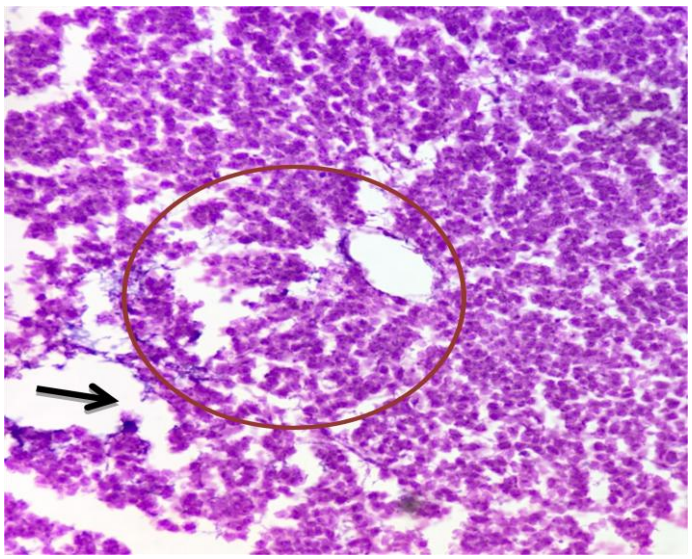

Figure: 2. Paracetamol treated Group: Centrilobular necrosis with changes pertains to Karryohexis (nuclear fragmentation) and Extremely Widened sinusoidal space.

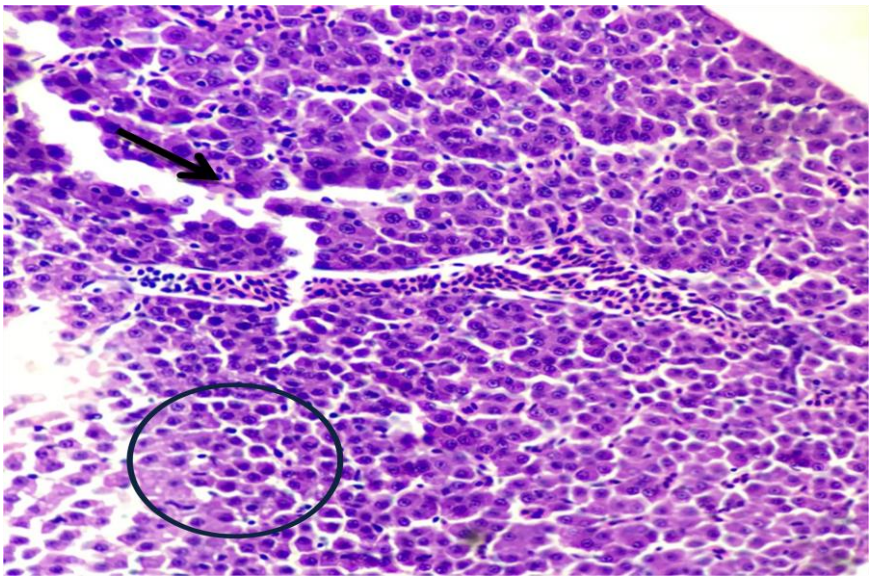

Fig: 3. KKC (250 mgm) treated Group: Widened sinusoidal space with Moderate necrotic changes.

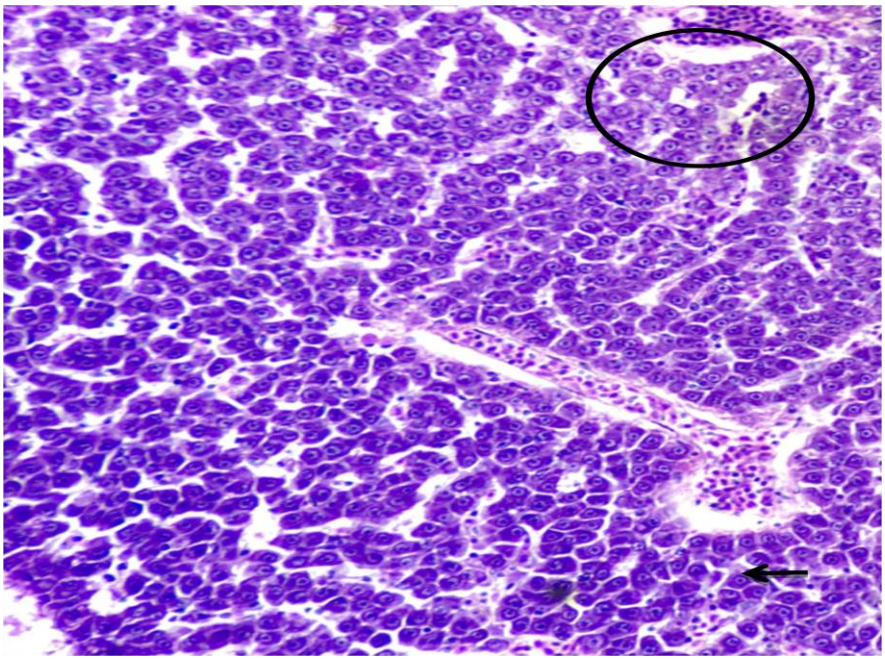

Fig: 3. KKC (500 mgm) treated Group: Normal Hepatocytes with Mild degenerative changes.

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