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EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF SIDDHA FORMULATION *PUNGAMPOO CHOORANAM AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN ZEBRAFISH MODEL*

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ABSTRACT

Liver plays a versatile role on detoxification and metabolism of xenobiotics. This could be the main reason that liver often prone to inflammation and degeneration. Management of liver disease becomes a major public threat in the developing countries. Conventional medication available for treating liver disorders fails to provide adequate cure and further in some cases it even worsen the condition. Hence it's a right time to explore alternate complimentary therapy for Indian system of traditional medicine such as siddha for effective management of clinical. Siddha medicines are pioneers in specific ailment for curative against liver disease since centuries. The main aim of the present investigation is to evaluate the hepatoprotective activity of the siddha formulation *Pungampoo Chooranam* (PPC) which majorly comprises of *Pongamia pinnata* in paracetamol induced liver dysfunction in zebrafish model. *Induction of hepatotoxicity in* Zebrafish was carried out by admixing paracetamol 5mM (755.8mg) per liter concentration. Zebrafish (*Danio rerio*) attains greater importance in the field reproductive biology due to it's close genetically resemblance with respect to that of the

humans. The results obtained from the present investigation has clearly reflects that the drug PPC at the concentration of 250 and 500 mg/liter has significantly ameliorated the Paracetamol induced hepatotoxicity in zebrafish model dose dependently. This study has provided an evidence based data on exploring the formulation PPC as a hepatoprotective agent for clinical management of liver disease.

KEYWORDS: Liver, Siddha, *Pungampoo Chooranam*, Paracetamol, Zebrafish, Hepatoprotective activity.

1. INTRODUCTION

Hepatic degeneration induced by chemicals and various drugs happens to be the most common type of iatrogenic disease, and the situation is further worsened by the absence of reliable and specific treatment.[1] The rate of morbidity and mortality due to hepatotoxicity or liver dysfunction is on the rise which makes it a major health problem throughout the world posing a big challenge to health-care professionals, drug regulatory agencies, and pharmaceutical industry to find an adequate, suitable treatment.[2]

Herbs become the integral part of the mankind since several centuries. India is considered to be one of the most significant zones for cultivation and export of the medicinal plant. According to the literature nearly 60–80% of the world's population still relies on traditional medicine. Indian system of traditional medicine such Ayurveda, siddha and unani formulation comprises greater of herbs or herbomineral combinations. There is always a constant demand for alternate herbal therapy because of known potential side effect caused by conventional allopathic drugs.[3]

Pongamia pinnata is one such Indian traditional herb which offers numerous therapeutic benefits to mankind since several centuries. Siddha system of traditional medicine uses several valuable medicinal herbs for its formulations. *Pongamia pinnata* (Fabaceae) is popularly known as Indian beech in English.[4] Commonly known by its vernacularnames karanj (Hindi), honge/karajata (Kannada), pungai (Tamil). As per the literature the extract of stem bark of *P. pinnata* (L.) showed antihyperglycaemic activity in diabetic mice.[5] Further, reports available that concomitant administration of synthetic oral hypoglycemic drugs along with *P. pinnata* produced synergistic effect in diabetic mice.[6] The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, triterpenes, flavonoids, steroids, and volatile oils.[7] It has been identified that Cycloart-23-ene-3*β*, 25-diol (B2)

isolated from the stem bark of *P. pinnata* possesses antidiabetic activity in diabetic animals.[8] Cycloart-23-ene-3*β*, 25-diol improved the abnormalities of diabetic conditions in diabetic mice due to increased glucagon-like peptide 1 (GLP-1) insulin secretion[9] and has a protective effect on vital organs like heart and kidney.[10] In search of finding the alternate therapy from siddha origin the present study aimed at investigating the hepato protective nature of the formulation PPC against paracetamol induced liver dysfunction in zebrafish model.

2. MATERIALS AND METHODS

2.1. Source of raw drugs

The herb is collected from southern zone of Tamil Nadu, and other required ingredient is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India .Herb were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India.

2.2. Ingredients

The siddha formulation *Pungampoo Chooranam* (PPC) comprises of two main ingredients as listed below

1. Pungam flowers (*Pongamiapinnata*)
2. Cow's Ghee

2.3. Preparation[11]

The shade dried flowers of *Pongamiapinnata* were roasted slowly by adding little bit of cow's ghee. Then it is powdered and sieved using cloth.

Dosage : 2 gm twice a day

Adjuvant : Warm water

Duration : 48 Days

2.4. Animal

Adult Zebra Fish (*Danio rerio*) 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.

2.5. Grouping

Group I – Control

Group II- Paracetamol 5mM (755.8mg) per liter concentration

Group III- Paracetamol 5mM + PPC Low Dose 250 mg/liter

Group IV- Paracetamol 5mM + PPC High Dose 500 mg/liter

2.6. Treatment[12]

Animal belongs to group I left untreated 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 received test drug *Pungampoo Chooranam* (PPC) at the concentration of 250 mg/liter and group IV received test drug *Pungampoo Chooranam* (PPC) at the concentration of 500 mg/liter along with paracetamol 5mM for the period of seven days.

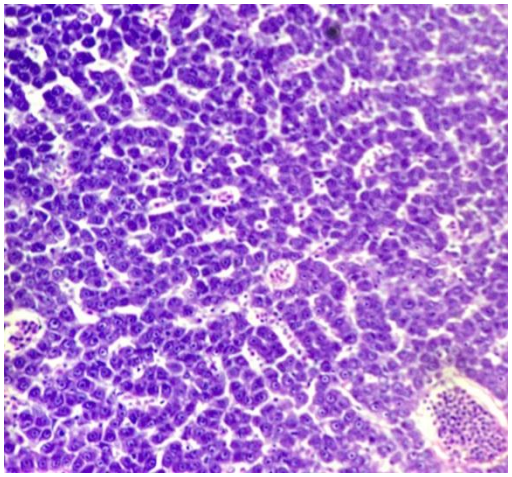
2.7. Histopathology[13]

After a one-week exposure period, the liver 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.

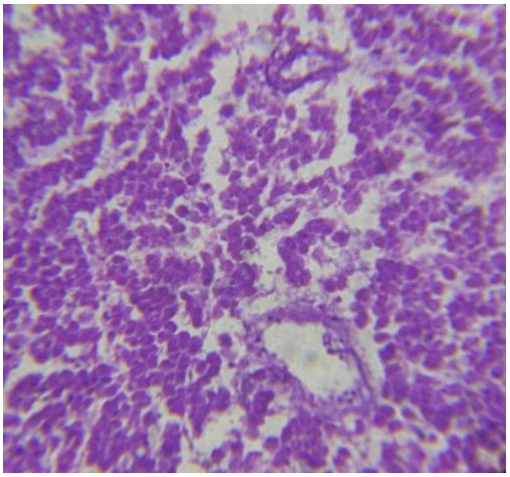
3. RESULTS

3.1. Effect of PPC on Liver Histology of Zebrafish

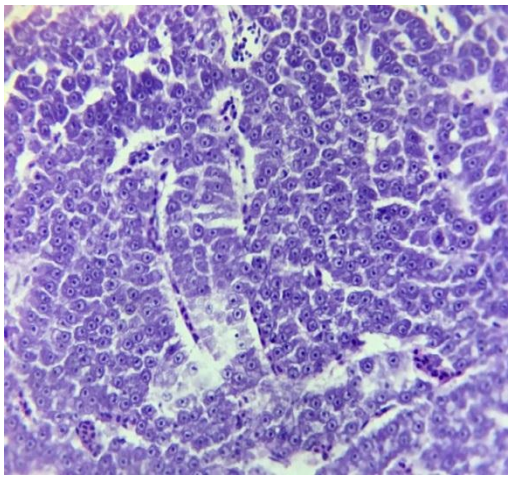
Light microscopy observation of the sample belongs to group I shows regular morphology with respect to architecture of hepatocytes. Liver sample belongs to group II reveals centrilobular necrosis with widened sinusoidal space. Histology sample belongs to group III reveals moderate necrotic changes with almost normal sinusoidal space. Sample belongs to group IV reveals almost normal morphology and with regular sinusoidal space. Treatment with PPC at both the dose level of 250 and 500 mg/liter has significantly ameliorated the paracetamol induced liver injury in a dose dependent manner.



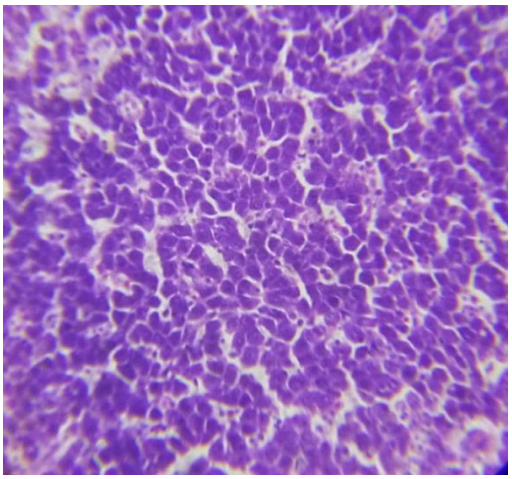
Group I – Normal Control



Group II – Disease Control



Group III – PPC Low dose (250 mg/liter)



Group IV –PPC High dose (500 mg/liter)

Figure 1: Effect of PPC on Liver Histology of Zebrafish against Paracetamol induced livery Injury.

4. DISCUSSION

Acetaminophen (APAP) also known by its name paracetamol is the most commonly used analgesic and antipyretic, which is relatively safe at recommended therapeutic doses.[14] However, its associated hepatotoxicity is a major concern and the leading cause of drug-induced liver failure in many countries when used at high doses.[15-16] Acetaminophen has been extensively studied in order to understand the mechanism of drug-induced hepatotoxicity.

Reported cases of APAP-induced hepatotoxicity first emerged in the United States in the mid-1980s, and since then all signs point towards a growing incidence. It has been reported that this is one of the most common pharmaceutical products to cause drug induced liver injury .Mortality rates have been approximated at 0.4% in overdose patients, translating to

300 deaths annually in the United States. Although toxic ingestions causing hepatic failure are usually in excess of 150 mg/kg, an increasing number of reports have arisen to suggest that lower doses of APAP may confer acute liver injury and liver failure.

There is a constant need of exploring alternate medicine from herbal origin for prevention and treatment of liver disease and research pertains to beneficial usage of herbs on liver disease management attains wider public attention because of the necessity is concern. Herbal medicine has been categorically employed for a variety of medical problems and modem trends have even helped in extracting the active principles which have been classed into many chemical groups such as alkaloids, glycosides, resins and tannins.[17] India is among the important mega biodiversity centers of the world with nearly 45,000 known plant species.[18] This diversity coupled with a rich heritage of traditional knowledge has made India home to several important health care systems viz., *Ayurveda*, *Siddha* and *Unani*.

A Zebrafish becomes a versatile high throughput model in toxicological and pharmacological evaluation of new drug entities and with advent lead molecules. It provides opportunity to carry out fast reproducible tests and high throughput behavioral screenings.[19-24] The zebrafish genome has approximately 70% homology with that of human and 84% of genes known to be associated with human disease have zebrafish counterparts.[25] The small size, rapid external development, optical transparency, less space and husbandry care and easy manipulation are few of the added advantages that play in its favor.[26]

In the present investigation Induction of hepatotoxicity in Zebrafish was carried out by admixing paracetamol 5mM (755.8mg) per liter concentration. Heptotoxic potential of paracetamol was evident by centrilobular necrosis with widened sinusoidal space. Treatment with PPC at both the dose level of 250 and 500 mg/liter has significantly ameliorated the paracetamol induced liver injury in a dose dependent manner. Liver sample belongs to group II reveals centrilobular necrosis with widened sinusoidal space. Histology sample belongs to group III reveals moderate necrotic changes with almost normal sinusoidal space. Sample belongs to group IV reveals almost normal morphology and with regular sinusoidal space.

5. CONCLUSION

The increasing use of herbal medicines reflects their perceived effectiveness in the treatment and prevention of disease, and the belief that these treatments are safe because they are ‘natural’. By proving this fact the data’s obtained from the present investigation clearly

projects that the siddha formulation PPC possess significant hepatoprotective activity and ameliorated the degenerative changes induced by paracetamol in zebrafish model. Hence it was concluded that the drug exerts promising hepatoprotective activity and may be considered as a drug of choice for treatment of liver disease.

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