INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 5, Issue 5 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2018.05.05.009

Evaluation of Hepatoprotective Potential of Classical *Siddha* Distillate *Sanjeevi Theeneer* against Paracetamol Induced Hepatotoxicity in Zebrafish (*Danio rerio*) Model

S.Vinayak[1*], R. Gayatri[2], S. Sivakumar[3], D.Sivaraman[4], Anil Sundaresan[5], V. Banumathi[6]

[1*]*Siddha* Physician, General Secretary, International Research Foundation for *Siddha* Science (INFOS), Kannur, Kerala.
[2]PG Scholar, Department of Noinadal, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India.
[3]Lecturer, Department of Gunapadam, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India.
[4]Scientist, Centre for Laboratory Animal Technology and Research, Col. Dr.Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Rajiv Gandhi Salai, Chennai 600 119, Tamil Nadu,
[5]Head of the Department & Chief Medical Officer, Department of Holistic Medicine, Apollo Clinic, Kannur, Kerala
[6]Director, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India.
Corresponding Author :**Dr. S. Vinayak,** Siddha Physician, General Secretary, International Research Foundation for Siddha Science (INFOS), Kannur, Kerala.
E-mail: drvinayak.sasv@gmail.com

Abstract

Background: Liver diseases are most common in clinical practice particularly due to chronic usage of certain medications such as analgesics leading to higher degree of liver injury and degeneration. On the other hand there are numerous herbal formulations with hepatoprotective activity in indigenous system of medicine like *Siddha*. Distillates prepared from Hepato protective herbs are the most promising among the formulations. **Objective:** The main aim of the study is to assess the hepato protective potential of classical *Siddha* polyherbomineral distillate, *Sanjeevi Theeneer* (ST) against paracetamol induced hepato toxicity in Zebra fish *Danio rerio* model. **Methods:** The studies have been conducted in 4 groups with 10 adult Zebrafish each. Group I being the control, Group II being the disease control group, Group II and IV being treated with test drug ST at the dose of 150 to 300 mg / litre concentration respectively**.** Histopathological assessment was done on the liver specimens after 7 days of drug exposure. **Results:** The result of the present investigation indicates that paracetamol treated groups shows severe liver degeneration whereas treatment with test drug ST at both the dose level significantly attenuated the paracetamol induced damage. **Conclusion:** This primary observation confirms the liver protective role of *Siddha* distillate, *Sanjeevi Theeneer* against paracetamol induced liver injury.

Keywords: *Siddha* distillate, *Sanjeevi Theeneer*, Paracetamol, Zebrafish, Hepato protective.

1. Introduction

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. Liver diseases are mainly caused due to over usage of certain drugs, alcohol and as an after effect of infectious diseases or autoimmune pathologies. Jaundice, liver cirrhosis and fatty liver are more prevalent in clinical practice especially in south India.[(1)] Out of this Jaundice and hepatitis accounts for the high mortality rates. [(2)]

As with concern of hepatotoxicity, drug-induced liver injury (DILI) poses a major clinical problem. DILI is initiated by direct hepatotoxic effects of a drug, or a reactive metabolite of a drug.

Paracetamol, a widely used analgesic and antipyretic drug, produces 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. The covalent binding of Nacetyl- P-benzoquinoneimine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver.[(2)]

In the other part, there are numerous herbal drug formulations with hepatoprotective effects[(3)] in indigenous system of medicine like *Siddha*. Distillates prepared from Hepato protective herbs are the most promising among the formulations. *Sanjeevi Theeneer* is a polyherbomineral classical *Siddha* distillate formulation effectively indicated for *Kamalai* (Jaundice and liver disorders) and for correcting blood parameters (Hemetenic). The drug is found to strengthen the functions of liver and spleen and further detoxifies blood.[(4)]

Many established animal models are available for evaluating hepatotoxicity versus hepatoprotectivity. As in vivo assessment provides high degree of accuracy and sensitivity as compared with *In vitro* studies primarily as it considers the whole system reflection of the drug effects moreover a dose dependent reactivity by the complex physiology of the whole organism can be studied much in depth and detail. [(5)] Using Lower order vertebrates like zebrafish is a good translational model in medical research because of numerous advantages including replacement of higher vertebrate organisms like rodents and Guinea pigs, cost effectiveness, shorter study period, easy drug delivery and husbandry and its astonishing similarity with that of human physiology. [(6)] Zebrafish (*Danio rerio*) is a very reliable model for studying drug induced toxicities in a variety of organ systems especially hepato toxicity.

Inspite difference in structural organization of liver tissue with that of mammalian liver, zebrafish shares similar physiology of drug metabolism as both share relative gene sequences for CYP enzymes (Cytochrome P450), the crucial drug metabolic enzymes present in liver. [(7)] As an eg, Hepato toxic drugs like Paracetamol is hydroxylated into N-acetyl-P-benzoquinone imine (NAPQ- Phase 1 reactive metabolite) by the same enzyme systems (CYP3A4) in both zebrafish and humans. [(8, 9)] Paracetamol causes hepatic necrosis in zebrafish liver which can be clearly depicted in the Histopathology specimens thereby establishing the feasibility to study the different histopathological changes in zebrafish liver through hepatotoxic drugs and hepatoprotective agents. [(10, 11)] The objective of the present study is to assess the Hepato protective effect of Classical *Siddha* polyherbomineral distillate, *Sanjeevi Theeneer* (ST) against paracetamol induced Hepato toxicity in Zebra fish *Danio rerio* model.

2. Materials and Methods

2.1. Preparation of the Test Drug

Sanjeevi Theeneer with reference to classical *siddha* texts has been selected for the study. All the 12 ingredients (Table 1) have been purchased from a reputed raw drug shop, after it was authenticated from the concerned department. The Raw drugs were purified, coarsely powdered and soaked in water under sterile environment over a period of 7 days. Further the contents were distilled in a traditional still to collect the distillate.

Table 1. Ingredients of *Sanjeevi Theeneer*[4]

S.No	Ingredient	Botanical Name	Quantity
1	*Chukku*	*Zingiber officinale*	120g
2	*Milagu*	*Piper nigrum*	120g
3	*Thippili*	*Piper longum*	20g
4	*Kadukkai*	*Terminalia chebula*	50g
5	*Nellikkai*	*Phyllanthus emblica*	50g
6	*Tantrikkai*	*Terminalia belerica*	50g
7	*Omam*	*Trachyspermum ammi*	50g
8	*Vaividangam*	*Embelia ribes*	50g
9	*Chithramoolam*	*Plumbago zeylanica*	60g
10	*Korai kizhangu*	*Cyperus rotundus*	50g
11	*Panam karkandu*	*Borassus flabellifer*	40g
12	*Irumbu Podi*	Purified Ferrum powder	120 g
13	Water		12 Litres

2.2. Animal

Zebrafish (*Danio rerio*) were used for the study

Purchase:	Local Aquarium facility.
Acclimatization:	Four weeks prior to the start of experimentation.
Laboratory condition:	28 °C ±1°C , 14:10 h light/dark cycle photo period.
Grouping:	Four groups of 10 fish each.
Weight per ml calculation of the drug	**ST = 0.015 gm (15000 micro gram or 15 mg)/ ml**
Drug administration:	Dilution of drug in water.
Drug Exposure period	7 days.

Table 2 Grouping of Animals for the study

Groups	Exposure	Period
I- Control	Untreated	7days
II- Toxicant	Paracetamol 5mM (755.8mg) per Litre concentration	7days
III- Test Group (ST Low Dose)	Paracetamol 5mM + ST Low Dose 150 mg/ Litre.	7days
IV- Test Group (ST High Dose)	Paracetamol 5mM + ST High Dose 300 mg/ Litre.	7days

2.3. Experimental Methodology

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 Sanjeevi Theeneer (ST) at the concentration of 150 mg/litre and group IV received test drug Sanjeevi Theeneer (ST) at the concentration of 300 mg/liter along with paracetamol 5mM for the period of seven days.

2.4. Histopathology

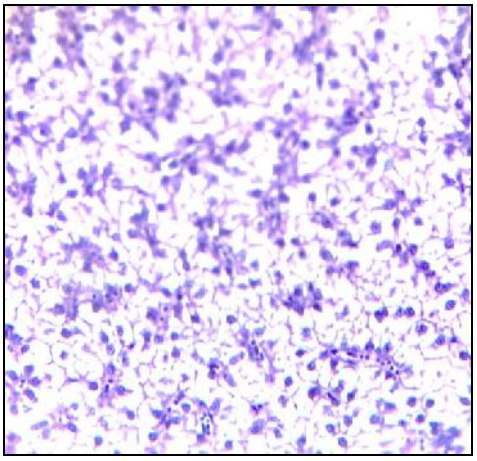
After a one-week exposure period, the livers of zebrafish were dissected and fixed in 10% formalin at 4 °C for 24h. Subsequently, the fixed liver tissues were dehydrated in gradient ethanol, hyalinized in xylene, and embedded in paraffin wax at 56 °C. Then, the paraffin blocks were sectioned at 4-µm 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

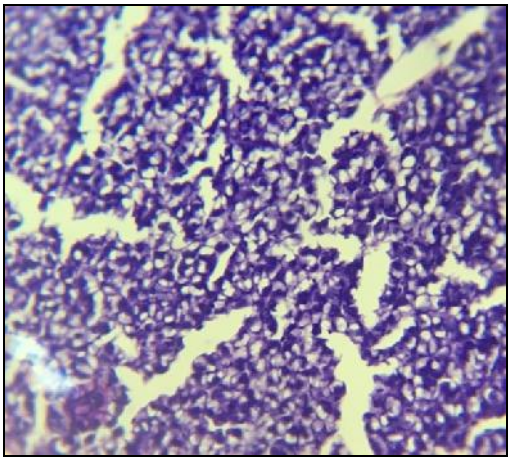
3.1. Effect of ST on Liver Histology of Paracetamol exposed Zebra fish Liver

Histopathology liver samples belong to control groups' reveals the presence of perfect polygonal shape of hepatic parenchyma cells with prominent nucleus further sinusoids appears with regular interval.

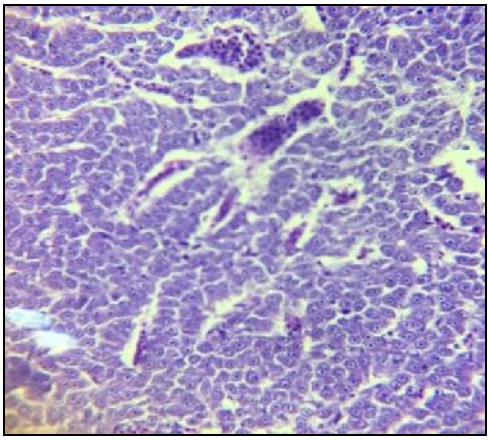
Figure1. Histo Pathology of liver section of Zebrafish (*Danio rerio*)



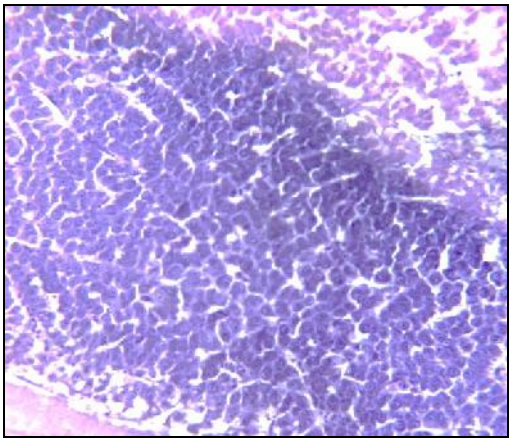
Group I : Control Group



Group II : Toxicant Group



Group III : Test Group (ST Low dose)



Group IV : Test Group (ST High dose)

Photomicrograph of liver section belongs to group II (Disease control) reveals the presence of frequent cytoplasm vacuolation and with abnormally dilated sinusoids. Sample belongs to group III retains the basic structure polygonal shaped hepatic parenchyma with occasional indication of karyorrhexis. Appearance of liver parenchyma was almost normal with prominent large nucleus with mild inflammatory changes were observed in sample belongs to Group IV. The results of the present investigation indicates that paracetamol treated groups shows severe liver degeneration whereas treatment with test drug ST at both the dose level significantly attenuated the paracetamol induced damage in group III and IV.

4. Discussion

In the modern day today life humans are exposed to several chemicals that are toxic to the liver. Most of the allopathic medications have threating side effects on liver hepatocytes if the dose and course of the medication is not followed wisely. Drug-induced liver injury is a major challenge in clinical medicine and drug development. New models are needed for predicting which potential therapeutic compounds will cause DILI in humans, and new markers and mediators of drug induce liver damage still need to be identified. Although the zebrafish liver architecture is different from that of the mammalian liver, the main physiological processes remain similar[12].

Since Phytomedicine has globally been the matter of interest in primary source of healthcare[13] that encouraged its utilization as a source of chemical diversity in drug development. Plant-derived medicines are known to have evolved under evolutionary pressure with diverse properties that make them suitable as lead structures in drug discovery [14]. Herbal medicines have also been recognized to provide specific substructures or scaffolds that make them comparable to trade drugs and their potential utilization in combinatorial chemistry[15]. Such exceptional properties exhibited by herbal derivatives

make their direct use in drug discovery as well as by using them as scaffolds to synthesize combinatorial repertoire proficient enough to bind against wide range of disease-specific targets. In fact, it could be argued that plants with medicinal values may have co-evolved with humans.

Siddha system of medicine offers numerous formulations with the advantage of hepatocellular rejuvenation. *Sanjeevi Theeneer* is one such classical preparation that is extracted from 11 novel traditional herbs. The liquid nature of the drug with the advantage of faster and extensive absorption render its pharmacological value in so many ailments.

Acetaminophen produces a centrilobular hepatic necrosis that can be fatal. Acetaminophen poisoning accounts for approximately one-half of all cases of acute liver failure in the United States and Great Britain today annually, it accounts for a very high percentage of inquiries to poison control centers and deaths.

Paracetamol induced liver injury becomes a global predictive model to ensure the hepatoprotective nature of the drug under investigation. In the present study zebrafish model has been adopted to evaluate the hepatoprotective potential of the formulation *Sanjeevi Theeneer*.. Zebrafish metabolize drugs using similar pathways to those in humans; they possess a wide range of cytochrome P450 enzymes that enable metabolic reactions including hydroxylation, conjugation, oxidation, demethylation and de-ethylation. Following exposure to a range of hepatotoxic drugs, the zebrafish liver develops histological patterns of injury comparable to those of mammalian liver, and biomarkers for liver injury can be quantified in the zebrafish circulation. The results of the present investigation clearly reflects that, treatment with ST at both dose level offers significant protection against paracetamol induced liver damage. It was further justified with the histological findings. Photomicrograph of liver section belongs to group II (Disease control) reveals the presence of frequent cytoplasm vacuolation and with abnormally dilated sinusoids. Sample belongs to group III retains the basic structure polygonal shaped hepatic parenchyma with occasional indication of karyorrhexis. Appearance of liver parenchyma was almost normal with prominent large nucleus with mild inflammatory changes were observed in sample belongs to Group IV.

The results of the present investigation indicates that paracetamol treated groups shows severe liver degeneration whereas treatment with test drug ST at both the dose level significantly attenuated the paracetamol induced damage in group III and IV.

Herbs have integrated power of healing though their versatile biologically active components such as alkaloids, Flavonoids, Terpenoids, Saponins etc. Indian traditional medicinal system like *Ayurveda*, *Siddha* and *Unani* has a very rich history of their effectiveness; modern research also acknowledged the importance of such medicine. Components present within the herb possess significant pharmacological activity and the formulations like ST has remarkable biological activity against drug induced live injury and may be considered as drug of choice for clinical management of liver disease in future.

5. Conclusion

From the results of the present study it was concluded that the drug *Sanjeevi Theeneer* (ST) possess 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.

Acknowledgments

I wish to acknowledge my thanks to The Noble research solutions, Chennai, Tamil Nadu, India for their analytical and technical assistance in publishing this research work.

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DOI: 10.22192/ijcrcps.2018.05.05.009

How to cite this article:
S.Vinayak, R. Gayatri, S. Sivakumar, D.Sivaraman, Anil Sundaresan, V. Banumathi. (2018). Evaluation of Hepatoprotective Potential of Classical *Siddha* Distillate *Sanjeevi Theeneer* against Paracetamol Induced Hepatotoxicity in Zebrafish (*Danio rerio*) Model. Int. J. Curr. Res. Chem. Pharm. Sci. 5(5): 44-49.
DOI: http://dx.doi.org/10.22192/ijcrcps.2018.05.05.009