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Research Article

SCIENTIFIC VALIDATION OF SASTRIC SIDDHA DRUG MANJAL KAMALAI KIYAZHAM AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN ZEBRA FISH (*DANIO RERIO*) MODEL

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ABSTRACT

Objective: The objective of this study is to assess the hepatoprotective nature of Siddha formulation manjal noi kudineer (MNK) against Paracetamol induced hepatotoxicity in Zebrafish *Danio rerio* model.

Methods: Pharmacological studies were carried out in the adult zebra fish model in the category of four groups; each individual group contained ten animals. Groups 1, 2, 3, and 4 were considered as control groups, disease control groups, and drug treated groups at different concentrations, respectively. Histopathological analysis was recorded.

Results: The observed research findings were confirmed that this novel Siddha formulation MNK possess potent hepatoprotective effect at the both dose level of 250 mg/l–500 mg/l against paracetamol induced toxicity in Zebrafish.

Conclusion: Siddha medicinal formulation has been in practice for more than 1000 years. Liver diseases are the highly prevalent one among all the diseases. Although huge drugs are available for liver diseases, there is a need for cost effectiveness drugs without any adverse effect. From the study, it was concluded that the drug MNK has promising hepatoprotective activity in dose-dependent manners and restores the basic liver architecture by means of its rejuvenating potential against paracetamol induced toxicity in Zebrafish model. We can strongly suggest that the Sastric Siddha drug MNK to treat various kinds of liver diseases such as liver cirrhosis and hepatic carcinoma. This research work may be highly beneficial to the people who suffered from various liver diseases if the drug will be given clinically.

Keywords: Siddha medicine, Poly herbal, Traditional, Sastric, Hepatoprotective, Kudineer.

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INTRODUCTION

In Siddha system of medicines, the diseases on the liver are referred as "Kalleeral noikal." Management of Viral hepatitis in the Siddha medical system is still a magical wonder by the way of its admirable therapeutic values. According to Siddha medical pathology the hepatic diseases are caused based on the abnormalities seen in the bodily Vital humors Pitham and Kapham. The formulations were made by Siddhars according to the concept of "Arusuvai." The prevalence of hepatic carcinoma, liver failure, cirrhosis of the liver, and viral hepatitis is seems to be very high. Globally, 2 million liver death accounts every year mainly because of liver cirrhosis, viral hepatitis, and hepatic carcinoma [1].

Liver metabolism plays a significant role in our day-to-day human life. Sometimes even the medications also give some trouble to our health. The liver might be considered as the most significant organ in drug harmfulness since it is practically mediated between the site of absorption and the systemic blood circulation and is a significant site of metabolism and disposal of unfamiliar substances; these highlights render it a favored objective for drug poisonousness. Drug-induced liver injury (DILI) therefore poses a major clinical problem. DILI is started by direct hepatotoxic impacts of a medication, or a receptive metabolite of a medication. Various plants having hepatoprotective property and those are considered as a very rich source to treat liver diseases [2]. Some herbals in Siddha medicine such as *Curcuma Longa*, *Wedelia Sinensis*, and *Terminalia chebula* claim high for its hepatoprotective effects. Likewise various herbo mineral and herbo metallic formulations were given by great Siddhars to treat different kinds of diseases on the liver without causing any adverse effects. Even though Sastric Siddha drugs are highly therapeutic in its action, preclinical and clinical evidences have to be investigated to explore its potent medicinal value all over the world. Drug induced toxicity can be easily evaluated in Zebrafish model [3].

Among many Siddha formulations, Manjal noi kudineer (MNK) is a best drug in the management of livers disorders. Although it was indicated in Sastric literatures, still there is no scientific background claiming its hepatoprotective effect. Here an attempt is made to evaluate the hepatoprotective effect of MNK in the Zebrafish model using paracetamol induced hepatotoxicity.

METHODS

The Siddha formulation MNK was prepared as per the Sastric Siddha book entitled [4]. The details of the ingredients are shown in Table 1.

Preparatory methods of drug sample MNK

Drugs were purchased from Earth India naturals, a Good Manufacturing Practices certified company. All the ingredients were taken into equal quantity, then dried and grinded into a coarse powder. With the use of water the test drug, MNK was prepared as per the Siddha texts. The images of the drug MNK raw drug and decoction were shown below in (Figs. 1 and 2).

Pharmacological–experimental section methodology

Animal

The laboratory Adult Zebrafish (*Danio rerio*) were purchased from the nearby aquarium and were maintained in a laboratory condition 28°C±1°C for a period of 14:10 h light/dark cycle photoperiod. All fishes were acclimatized to standard lab condition 4 weeks before the start of experimentation. Animals were divided into four groups of ten fish

Table 1: Details of herbs formulated in Siddha drug MNK

Sl.no	Plant name (Tamil name)	Parts used	Botanical name	Family name	Biological property
1	Keezhkaai Nelliver	Root	*Phyllanthus amarus*	*Phyllanthaceae*	1. Hepatoprotective effect [5] 2. Anti-oxidant activity [6] 3. Growth inhibitory activity on cancer cell lines [7] 4. Increase lifespan on Hepatocellular carcinoma [8]
2	Sirunerinjil Vaer	Root	*Tribulus terrestris*	*Zygophyllaceae*	1. Hepatoprotective [9-11] 2. Antioxidant activity [12].
3	Seeragam	Dried fruits	*Cuminum cyminum*	*Apiaceae*	1. Anti-inflammatory effect [13] 2. Antioxidant activity [14] 3. Hepatoprotective activity [15] 4. Anticancer activity [16]
4	Pei Pudal	whole plant	*Trichosanthes dioica*	*Cucurbitaceae*	1. Immunomodulating effect [17] 2. Hepatoprotective effect [18] 3. Antioxidant activity [19]
5	Vilvamver	Root	*Aegle marmelos*	*Rutaceae*	1. Anticancer Effect [20] 2. Hepatoprotective activity [21] 3. Antioxidant activity [22,23]
6	Sirukeerai Ver	Root	*Amaranthus tricolor*	*Amaranthaceae*	1. Hepatoprotective Activity [24] 2. Anticancer Effect [25] 3. Antimicrobial and antioxidant activities [26,27]
7	Karisalankanni	whole plant	*Eclipta prostrata*	*Asteraceae*	1. Hepatoprotective activity [28,29] 2. Anticancer activity [30] 3. Antifibrotic effects [31] 4. Antimicrobial activity [32] 5. Antiproliferative activity [33]
8	Valmilagu	Seed	*Piper nigrum*	*Piperaceae*	1. Immunomodulatory effect [34] 2. Antihepatotoxic effect [35] 3. Antioxidant effect [36]
9	Sombu	Dried fruits	*Foeniculum vulgare*	*Apiaceae*	1. Antioxidant effect, anticarcinogenic effect [37] 2. Hepatoprotective effect [38,39].

MNK: Manjal noei kudineer

each. The weight/ml calculation of the drug MNK=0.050 gm (5000 mg or 50 mg)/ml.

Grouping

- Group I: Control
- Group II: Paracetamol 5 mM (755.8 mg) per liter concentration
- Group III: Paracetamol 5 mM+MNK Low Dose 250 mg/l
- Group IV: Paracetamol 5 mM+MNK High Dose 500 mg/l.

Treatment

Animal belongs to Group I left untreated and Group II treated with Paracetamol at the concentration of 5 mM (755.8 mg) per liter concentration for the period of 7 days. Animal belongs to Group III received test drug MNK at the concentration of 250 mg /l and Group IV received test drug MNK at the concentration of 500 mg/l along with paracetamol 5 mM for the period of 7 days.

Histopathology

After a 1-week exposure period, fishe were sacrificed and the livers of Zebrafish were dissected and fixed in 10% formalin at 4°C for 24 h. Subsequently, the fixed liver tissues were dehydrated in gradient ethanol, hyalinized in xylene, and embedded in paraffin wax at 56°C. At that point, the paraffin blocks were separated at 4-µm thickness. The segments were gathered on glass slides and recolored with hematoxylin and eosin (H and E) utilizing a H and E Staining Kit. Histologic lesions were watched utilizing an optical microscope instrument outfitted with a computerized camera.

RESULTS AND DISCUSSION

The histological microscopy findings belong to the normal control Group I (Fig. 3) show the presence of regularly arranged hepatocytes exist in polygonal shape cells and with prominent nucleus further sinusoids appears with regular intervals. In Group II (Fig. 4), severe discrete

Fig. 1: Manjal noi kudineer-raw drug

cytoplasmic vacuoles and rare foamy cytoplasm were observed. This prominence indicates the presence of inflammatory changes in it. The histopathological findings of Group III (Fig. 5) retain the basic structure of polygonal shaped hepatic parenchyma with occasional Pyknotic nuclei and of Group IV (Fig. 6) shows that regenerated liver cells with Occasional bi nucleated hepatocytes were observed. This is due to the hepatoprotective nature of the drug MNK treated. The reasons behind the hepatoprotective effect of this formulation are possibly due to the presence of herbals in it. One pharmacological research findings confirms that the aqueous extract of *Phyllanthus niruri* exhibits hepatoprotective effects against carbon tetrachloride induced liver toxicity in mice. The herbals *T. terrestris* and *Bauhinia acuminata* present in this formulation were already documented for its immunomodulatory effects. In addition

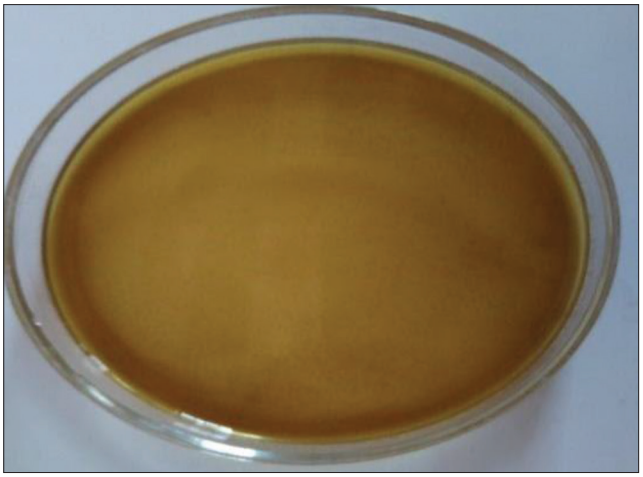

Fig. 2: Manjal noi kudineer-kudineer decoction

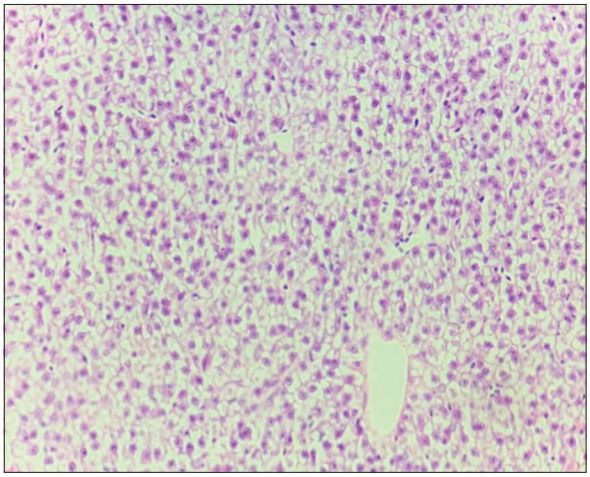

Fig. 3: Group I (Normal Control)

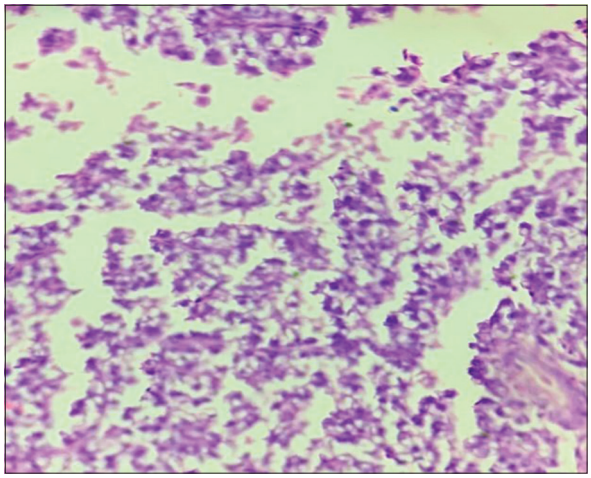

Fig. 4: Group II (Disease Control)

to that the other herbal ingredients present in this formulation have anti-inflammatory, antioxidant activity was identified through pharmacological action. All these beneficial effects of this plant significantly may have therapeutic values to treat hepatic diseases. The flavonoids and other phytochemical constituents present in the herbals of this formulation may be the factors behind the hepatoprotective effect of this siddha drug MNK.

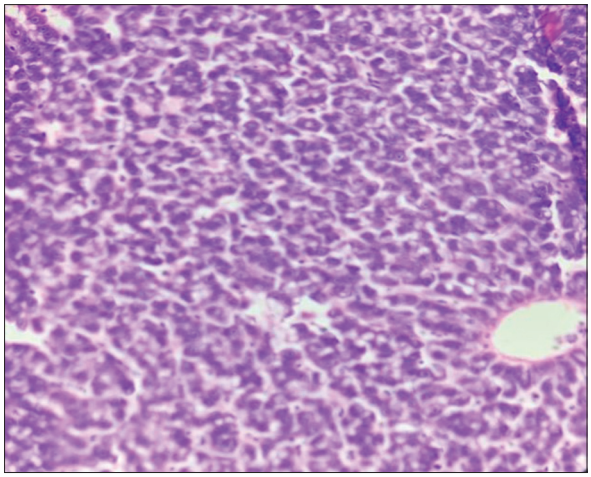

Fig. 5: Group III (Low dose of manjal noi kudineer treatment)

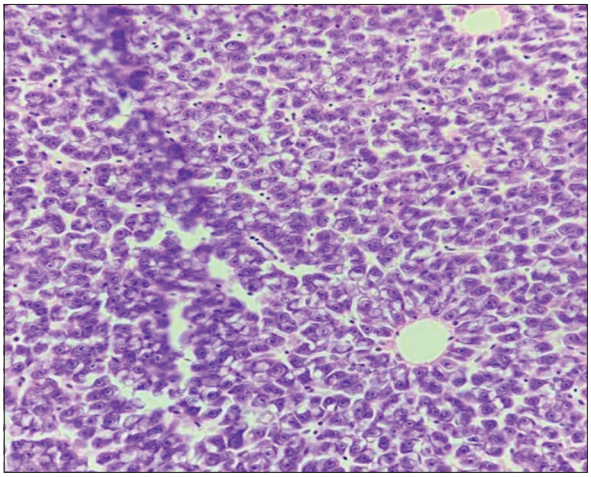

Fig. 6: Group IV (High dose of manjal noi kudineer treatment)

CONCLUSION

The results of the present investigation indicate that paracetamol treated groups show severe liver degeneration and inflammation associated fibrosis whereas treatment with test drug MNK at both the dose levels fundamentally constricted the paracetamol prompted harm in Groups III and IV. Hence, from the study, it was concluded that the drug MNK has promising hepatoprotective activity in dose-dependent manners and restores the basic liver architecture by means of its rejuvenating potential against paracetamol induced toxicity in Zebrafish model. We can strongly suggest that the Sastric Siddha drug MNK to treat various kinds of liver diseases such as liver cirrhosis and hepatic carcinoma; however, further study is required to identify the hepatoprotective constituents present in the formulation as well as elucidating the mechanism of action.

CONFLICTS OF INTEREST STATEMENT

No conflicts of interest.

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Nil.

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AUTHOR CONTRIBUTION

This study was designed, directed, and coordinated by principal investigator provided conceptual and technical guidance for all aspects of the project by Dr. R. Gomathi, Dr. E. Preetheekha, Dr. P. Shanmuga Priya, and Dr. A. Mamallan Suggested and commented on the design of the experiments.

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