EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF MUPPU, A SIDDHA PREPARATION, IN RATS

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Summary

The present study was undertaken to carryout the determination of hepatoprotective activity of mineral mixture extract, muppu, in carbon tetrachloride induced intoxication in rats. The muppu extract at an oral dose of 50 mg/kg exhibited a significant ($P < 0.001$) protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatise and total bilirubin.as compared to hepatotoxic control. These biochemical observations were supplemented by histopathological examination of liver sections. Thus it could be concluded that extract of muppu possesses significant hepatoprotective properties. However, further confirmations are needed to detect active principles of muppu responsible for hepatoprotective activity.

Key words: Muppu; Carbon tetrachloride; Siddha; hepatoprotective; Liv-52.

Introduction

Siddha is a traditional medical system of India. It is of Dravidian origin and has its entire literature in Tamil language. Its origin is also traced to mythological sources belonging to the ancient tradition. Siddha system of medicine is used to cure fungal infections, cough, cold, diarrhea and fever. Siddha system is very effective in treating ailments such as varieties of joint diseases, skin diseases, liver problems and urinary tract infections. They are also known for rejuvenative, antioxidant and adaptogenic properties. In Siddha medicine the use of metals and minerals are more predominant in comparison to other Indian traditional medicine systems. The use of more metals and chemicals was justified by the fact that to preserve the body from decomposing materials that do not decompose easily should be used. The other reason perhaps was that the south Indian rivers were not perennial and herbs were not available all through the year. One of such preparation is muppu [1].

Muppu has significant role in siddha system of medicine. These are preparations used for making mercury pills of very high potency. The various kinds of muppu preparations are reported such as vaidya muppu or the medicinal one,vada muppu- employed in South India alchemy; yoga muppu - meant exclusively for spiritual aspirants; jnana muppu - meant exclusively for spiritual aspirants and other types. Three ingredients are usually added into the preparation of medicinal muppu they are sodium carbonate (puniru), rock salt (kaluppu) and calcium carbonate (andakkal). This makes the mercury attains its efficiency and therefore, it is described as "guru". Muppu is used in folklore for rejuvenative and antioxidant properties [2]. The aim of the present study was to evaluate potency of muppu in providing hepatoprotective activity in presence of hepatoxicity induced by carbon tetrachloride in rats.

Materials and Methods

Preparation of muppu

Muppu is a combination of three kinds of salts prepared as per the palm leaf literature described in Kandarnadi Vaakiyam. These salts are processed from three different sources obtained in Tamil Nadu, India. The first salt is called vediuppul/shivappu obtained from rocky salt formation in seashores of Trichundur district of Tamil Nadu. The second salt is known as brahmuppu, obtained from Kanyakumari district of Tamil Nadu. The third salt is procured by insertion of bamboo sticks in deep sea at a location where three oceans meet. All the three salts are processed from the respective materials by extracting with amuri (specially prepared juice from banana tree) and purified as per procedures described in palm leaf literature. Approximately 50 mg/kg body weight was used traditionally in human for various hepatic ailments. Hence the same dose was selected in the current study without evaluation of toxicity.

Experimental animals

Laboratory bred (256) female Wistar albino rats (200-250 g) were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Carbon tetrachloride-induced hepatotoxicity in rats

Rats were divided into four groups of six each, control, hepatotoxin, positive control and test groups. The control group received oral vehicle treatment at 0, 24 and 48 h. The animals in hepatotoxin-treated group received vehicle at 0 h and at 24 h vehicle followed by carbon tetrachloride diluted in liquid paraffin (1:1, i.p.) at a dose of 1.25 ml/kg, while at 48 h these animals received only vehicle. The test groups have received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of carbon tetrachloride and at 48 h the third dose of extracts[3,4]. The positive control group has received the first dose of liv.52 (0.125 mg/kg body wt) [5] at 0 h, at 24 h the second dose of liv.52 followed by a dose of carbon tetrachloride and at 48 h the third dose of liv.52. After 72 h blood was collected from all the groups, and allowed to clot for the separation of serum. The serum was used for estimation of biochemical parameters.

Glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) [6], alkaline phosphatase (ALKP)[7] and total bilirubin (TBL) were estimated [8]. All the determinations were carried out using standard kits by an semi autoanalyser of Glaxosmith.

Histopathological studies

At the end of experiment, rats are sacrificed under over dose of diethyl ether, abdomen was cut open, liver was excised out and weighed. One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 h, and then embedded in paraffin using conventional methods [9] and cut into 5μm thick sections and stained using haematoxylin–eosin dye and finally mounted in di-phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture.

Statistical analysis

The mean values±SEM are calculated for each parameter. For determining the significant inter-group difference each parameter was analyzed separately and one-way analysis of variance (ANOVA) [10] was carried out and the individual comparisons of the group mean values were done using Dunnet's Procedure [11].

Results

Carbon tetrachloride (CCl_4) intoxication in normal rats elevated the levels of SGOT, SGPT, ALK and TBL significantly indicating acute hepato cellular damage. The rats treated with muppu extract and also liv-52, showed a significant decrease in all the elevated SGOT, SGPT, ALKP and TBL levels (**Table 1**). There was significant incline in the weight of liver in CCl_4 intoxicated rats, whereas, upon treatment with either muppu or liv-52 in CCl_4 intoxicated rats, there was significant decline in liver weight. Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. Disarrangement of normal hepatic cells with intense centrilobular necrosis and vacuolization of periportal vein are observed in CCl_4-intoxicated liver. The liver sections of the rat treated with muppu ethanolic extract and intoxicated with CCl_4, showed less vacuole formation and absence of necrosis and overall no visible changes observed as compared to liv-52, supplementing the protective effect of the extract.

Table 1- Effect of Muppu on carbon tetrachloride induced toxicity in rats

Treatment	Liver Weight (g)	Total Bilirubin (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)
Control	2.13±0.09	0.76±0.030	39.38±0.46	62.45±5.24	84.78±0.96
Carbon tetrachloride	4.38±0.28	13.59±1.11	486.03±8.42	370.7±13.38	424.65±12.09
Liv-52	2.96±0.09***	2.45±0.11***	276.5±5.71***	106.43±2.73***	247.83±9.58***
Muppu	3.33±0.20**	2.79±0.28***	304.2±2.29***	208.31±2.03***	335.61±10.09***

Values are expressed as mean ± SEM for eight rats in each group. ***Significantly different in treated group when compared to carbon tetrachloride group $P< 0.001$.

Discussion

The use of metals, minerals particularly mercury in medicine, which was a part of Tantric legacy is seen in all ancient Indian medical schools. This legacy is conspicuous in the so-called Siddha system of medicine, which is now prevalent mostly in South India, especially on Tamil Nadu. This system dating back to pre Ayurvedic period which is of strong Tantric in orientation. It got mixed up with the cult of 'navakoti siddhas' i.e., nine million Siddhas, who transcended the death, preached a philosophy of transmuting the gross physical body composed of impure matter into the refined body of naturally pure matter, thereby making the body immutable and free from disabilities and limitations. In the present study one such mineral mixture muppu extract was taken for the study. The extract of muppu possesses significant ($P < 0.001$) hepatoprotective effect in the CCl_4 model of intoxication in rats.

The hepatotoxicity of CCl_4 has been reported to be due to the formation of the highly reactive trichloro free radical, which attacks polyunsaturated fatty acids. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals[12]. The effect of CCl_4 is generally observed after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl_4 intoxication. From Table 1 it is evident that the extract was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localised in the endoplasmic reticulum which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides.

Reduction in the levels of SGOT and SGPT towards the normal value is an indication of stabilisation of plasma membrane as well as repair of hepatic tissue damages caused by CCl_4. Reduction of ALKP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl_4.

The protective effect exhibited by the extract is similar to liv-52 treatment. Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the sections obtained from the rats treated with extract and intoxicated with hepatotoxin, the normal cellular architecture was retained as compared to liv-52, there by confirming the protective effect of the extract. It can be concluded from this investigation that the muppu extract possess hepatoprotective activity against CCl4 intoxication in rats. Our further detailed studies may, however, confirm the utility profile of this mineral mixture.

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