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Hepatoprotective Activity of *Chara Parpam* in Ccl_4 Induced Rats

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Abstract: *Siddha system of medicine provides most frequently and to the extent possible and promising therapy for the relief of signs and symptoms of liver disorder over the generations. Their high therapeutic quality and lack of toxicity are exceptional. The present experimental work was to evaluate the hepatoprotective properties of Siddha herbo-mineral formulation Chara Parpam by CCl_4 induced hepatotoxicity in albino rats. Two doses of Chara Parpam (5 mg/kg and 10 mg/kg) were administered to rats. Protection of hepatocytes was evaluated by estimate the level of ALT, AST, ALP, serum bilirubin, total protein, serum albumin, sodium and potassium during the exposure of CCL_4 on wistar albino rats and to evaluate the effect of different doses of Chara Parpam against hepatotoxicity induced by CCL_4. Liver histology was performed 24 hours after the administration of trial drug Chara Parpam. The result indicated that the concentration of ALT, AST, and ALP, released by hepatocytes were significantly reduced in the presence of Chara Parpam. The cytoprotective effects of the Chara Parpam are dose-dependent. Through this work, we demonstrate for the first time the direct protection of liver cells by administration of Chara Parpam confirming its hepatoprotective properties.*

Key words: *CCl_4, Chara Parpam, hepatoprotective activity, histopathology, Serum transaminase, Siddha Medicine*

I. Introduction

The liver is one of the largest and most important internal organ of the human body and considered as a chemical factory which performs more than 500 vital function. Its main functions include clearance, biotransformation and detoxification of potentially toxic metabolites and exogenous compounds, synthesis and export of various plasma proteins, and a critical integrative role in the intermediary metabolism of carbohydrates, amino acids, and lipids [1].

Because of the great importance of the liver, liver disorders have severely and negatively impact the quality of life and life expectancy. Most of the liver diseases can be caused by the exposure toxic substances, metabolic disorders, viruses and genetic abnormalities. The liver diseases constitute a class of the most prevalent chronic disease in India. The pathological process causing jaundice and other liver diseases are haemolytic, congenital, toxic, infective, and obstructive etc [2].

In spite of the tremendous advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells [3]. Siddha system of medicine provides most frequently and to the extent possible and promising therapy for the relief of signs and symptoms of liver disorder over the generations. Their high therapeutic quality and lack of toxicity are exceptional.

One such drug mentioned in the Siddha classical text to treat liver disorder is *Chara Parpam* [4]. The *Chara Parpam* is a well known herbo mineral formulation and is used frequently for its hepato-protective potential. It also strengthens the liver in its functions. But their hepatoprotective effects, mode of action, dosage and toxicity have not been so far scientifically established.

Hence, the present study is to evaluate the hepatoprotective effect of *Chara Parpam* by estimate the level of ALT, AST, ALP, serum bilirubin, total protein, serum albumin, sodium and potassium during the exposure of CCL_4 on Wistar albino rats and to evaluate the effect of different doses of *Chara Parpam* against hepatotoxicity induced by CCL_4. Carbon tetrachloride is a potent hepatotoxin, and a single exposure to it can rapidly lead to an increase in the level of several enzymes, severe centrizonal necrosis and steatosis [5]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [6, 7].

II. Materials And Methods

2.1. Preparation of *Chara Parpam*:

Chara Parpam was prepared by Siddha classical method and standard operating procedure was adopted. The preparation was carried out after fulfilling the following steps.

The ingredients of *Chara Parpam* are *Navacharam* (Ammonium chloride), *Vediyuppu* (Potassium nitrate) and the plant *Adathodai (Justicia adhatoda*). The raw drugs Ammonium chloride and Potassium nitrate were purchased from TAMPCOL, Arumbakkam, Chennai. The plant *Justicia adhatoda* was collected from in

and around Chennai. Both the raw materials were identified and authenticated by the experts of Gunapadam and the plant was identified and authenticated by the botanist, Govt.Siddha Medical College, Arumbakkam, Chennai. The voucher specimens of each sample have been kept in the department of further reference.

2.2. Purification of raw drugs

The raw drugs *Navacharam* and *Vediyuppu* were purified as per the Siddha classical literature [8]. The *Chara Parpam* was prepared as per the "*Pathartha Guna Vilakkam* part-II [9]. The procedure for the preparation of formulation as described in the literature was strictly followed and was standardized.

2.3. Method of Preparation:

Ammonium chloride and Potassium nitrate kept in a *Kalvam* (Stone mortar with pestle) separately and finely powdered. Both the powders of raw materials mixed together and grounded with juice of *Justicia adhatoda* (Photo.1) for 12 hours and made in small cakes (*Villai*) and dried in sunlight. These cakes were then kept in a suitable mud pan and sealed with 7 layers of clay cloth (*Seelai mann*). Then the sealed vessel (*Kavasam*) was dried under sunlight. After complete drying, this sealed vessel was burned for 12 hours. Allow it to cool at room temperatures then the sealed vessel was opened and collected the drug, which was white in colour (Photo.2). This drug was powdered well in Mortar with pestle and termed as *Chara Parpam* (Photo.3) and used for further study.

Photo.1. Juice of *Justicia adhatoda*

Photo.2. Cakes of *Chara Parpam*

Photo.3. Final product of *Chara Parpam*

2.4. Animals

Wistar albino rats (150-200 g) and Mice of either sex weighing 25-30g were procured from animal housing facility of School of Pharmaceutical Sciences, Vels University, Chennai. The animals were housed in well ventilated large hygienic spacious cage and animals had 12 hours day and night schedule with temperature between 28 ±20C. The animals were allowed free access to standard laboratory pellets and drinking water *ad libitum*. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (Approval number: XIII/ VELS/ PCOL/ 01/ 2000/ CPCSEA/ IAEC/ 08.08.2012).

2.5. Chemicals

Estimation Kits for AST, ALT, ALP, T.P, etc were obtained from SPAN diagnostics and Standard drug Silymarin and hepatotoxin CCL_4 (Sigma-aldrich chemical Pvt. Ltd., Bangalore.) were used in the present study.

2.6. Determination of acute oral toxicity

Acute toxicity of *Chara Parpam* was done according to the OECD guidelines No.425 [10]. The overnight fasted mice were given in various doses (2000, 1000, 500, 250, 100 and 50mg/kg b.w.), the animal were observed continuously for the first two hours and at 24 hours to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were monitored up to 14 days for the toxic symptoms and mortality.

2.7. Evaluation of Hepatoprotective activity

CCL_4 induced hepatotoxicity in rats model [11] was used for evaluation of hepato-protective activity for the *Chara Parpam*. Animals were divided into five groups, each group containing six animals.

Group I (normal) - received 2% CMC for 14 days.

Group II (Control) - received CCl_4 1ml/kg, i. p. 1:1 dilution with coconut oil on 5th day.

Groups III and IV - received *Chara Parpam* (5mg/kg and 10mg/kg p.o) for 14 days and CCl_4 induction on 5th day.

Group V - received standard drug Silymarin (25mg/kg per day, p.o.) for 14 days and CCl_4 induction on 5th day.

After 14 days of experimental period blood sample had been collected individually for all the animals by retro-orbital puncture method and the blood was allowed to clot for 30 min; serum was separated by centrifuging and was used for various parameter estimations. Later all, the animals were sacrificed by cervical dislocation, liver samples were collected and the individual weights of the livers were estimated. For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formalin in saline.

2.8. Biochemical parameters studied

The activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) [12] were estimated using standard methods. Estimation of serum ALP [13], serum bilirubin [14] and electrolytes were also carried out to assess the acute hepatic damage caused by CCl_4.

III. Statistical Analysis

The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Dunnet's test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using INSTAT- V3 Software programme.

IV. Result And Discussion

4.1. Acute Toxicity

The results of acute oral toxicity study suggested that the *Chara Parpam* was safe up to 50mg/kg. As per the above study, dose fixation was done and 5mg/kg and 10mg/kg have been selected as low and high doses respectively for the hepatoprotective study.

4.2. Hepatoprotective study

The result of Table No.1 and Fig No.1 showed that administration of *Chara Parpam* has variations on liver weight in experimental rats with liver damage induced by CCL_4. As there was significant increase in liver weight due to damage caused by administration of CCL_4 in control animals as compared to normal group [15]. In animals treated with Silymarin and *Chara Parpam*, there was significant reduction in liver weight compared to toxic group.

Fatty change or fatty liver is the main consequence of liver toxicity which is characterized by the deposition of fat in liver. Due to the deposition of fat and triglycerides, total weight of liver increases in drug induced hepatic damage. In positive control group, there was significant rise in liver weights but silymarin and *Chara Parpam* could able to normalize weight of livers in therapeutic groups by decreasing toxicity of liver.

The result of Table No.2. showed that CCL_4 administration caused significant elevation of ALT, AST,ALP, direct bilirubin and total bilirubin [16] by inducing hepatic damage in control animals as compared to normal animals while, standard drug silymarin treatment reduced ALT, AST, ALP, direct bilirubin and total bilirubin concentration in animals of standard group and those were almost equivalent to normal. Even though experimental animals pre - treated with trial drug *Chara Parpam* at both doses (CP – 5 mg and CP – 10mg) significantly decreased the ALT, AST, ALP, direct bilirubin and total bilirubin level, there was a dose dependent significant reduction in liver panel levels in *Chara Parpam* (CP – 10mg) treated animals as compared to control group. In positive control group animals treated with CCL_4, there was significant decrease in serum total protein, albumin and serum ionic concentration due to liver damage when compared to saline alone treated animals but administration of silymarin caused significant rise in total protein compared to control group. In animals administered with *Chara Parpam* there was significant increase total protein level and it was dose dependent. Histological profile of the control animals showed normal hepatocytes.

Liver is important storage organ and stores various serum enzymes like ALT and AST which are involved in transamination reactions for various amino acids. Alkaline phosphatase is isoenzyme synthesized mainly by liver and has vital role in dephosphorylation of various biomolecules. In liver disease, liver cannot store ALT and AST and increased synthesis of ALP observed due to liver parenchymal damage caused by liver toxicity. Among various, one of the important functions of liver is detoxification of bilirubin which is breakdown product of haem. The bilirubin uptake by liver parenchyma cells from the blood and conjugates with glucuronic acid in presence of enzyme glucuronyl transferase, later conjugated product excreted into bile.

In liver toxicity total bilirubin and direct bilirubin concentration in serum increases due to abnormality in hepatic parenchymal cells. In our present study, CCL_4 elevated levels of ALT, AST, ALP, direct bilirubin and total bilirubin was observed in control animals which may be due to reduced function of liver due to toxicity. Treatment with silymarin and *Chara Parpam* significantly reduced concentration of above serum parameters in animals of therapeutic groups which could be due to possible protection given by *Chara Parpam*.

The albumin and globulin are the main components of total protein in the plasma and albumin mainly synthesized by the liver. In CCL_4 induced hepatotoxicity, albumin synthesis will be decreased due to cirrhosis and leads to reduction in total protein. Hence serum albumin and total protein are the two important biomarkers of liver function.

The ascites and edema are the two main complications of liver injury which is due to accumulation of fluids in extravascular sites of the body and hence serum sodium and potassium moves into fluids which finally lead to reduced concentration of these ions in blood. In our present study in control animals treated with CCl_4, there was significant reduction in concentration of serum albumin, total protein, serum sodium and potassium due to toxicity. In therapeutic animals treated with standard drug silymarin and *Chara Parpam* significant increase in serum albumin, total protein, serum sodium and potassium was observed as compared to toxic group. Histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of *Chara Parpam* (Fig No.5). There is a cleavage of carbon tetrachloride leading to the formation of free radicals, which causes steatosis, cetrilobular necrosis and cytoplasmic vacuolation were observed in toxic control group. But these pathological changes were moderately prevented by the trial drug treated group and standard group.

Normal hepatic architecture similar to normal group was observed in the silymarin treated group (Fig No.5 C). While trial drug *Chara Parpam* 10 mg administered group also showed normal hepatic architecture with less infiltration of fat and absence of necrosis (Fig No. 5 E). It is evident that the *Chara Parpam* caused regeneration of liver parenchyma cells and treated hepatic cell damage due to CCL_4 toxicity.

Table 1: Effect of *Chara Parpam* on liver weight

GROUP	LIVER WEIGHT (gm)
Normal Saline	5.76± 0.10
Positive Control	8.42±0.68
Chara Parpam 5mg/kg	6.54±0.14*
Chara Parpam 10mg/kg	5.66±0.10**
Silymarin 25mg/kg	4.2±0.04**

*Values are mean ± S.E.M, n=6; $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$ Vs positive control.*

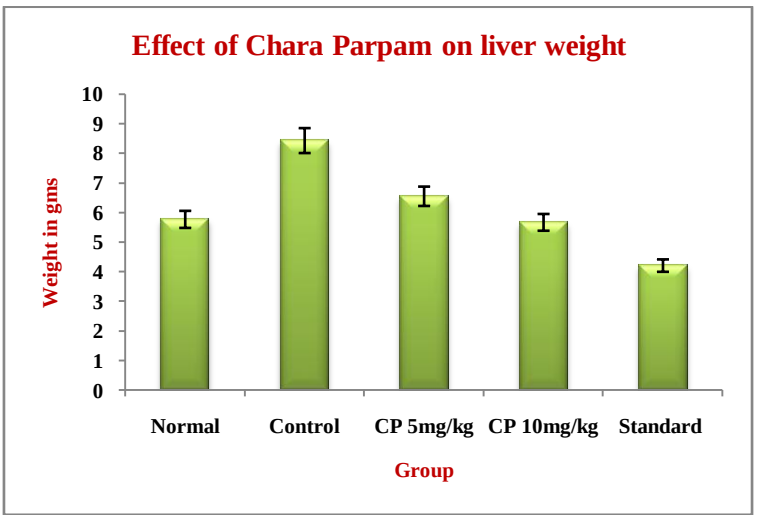


Fig No.1. Showing the effect of *Chara Parpam* on liver weight

Table 2: Effect of *Chara Parpam* on serum biochemical parameters

GROUP	ALT IU/L	AST IU/L	ALP IU/L	T. Bilirubin g/l	D. Bilirubin g/dl
Normal Saline	58.21±1.6	112.5±0.52	78.32±1.8	0.36±0.02	0.022±0.010
Positive Control	176.42±26.1	239.2±3.3	465.16±4.05	0.92± 0.02	0.28± 0.03
Chara Parpam 5mg/kg	98.33±2.8**	184.22±1.4**	262.01±2.0**	0.64±0.018**	0.12±0.004**
Chara Parpam 10mg/kg	72.59±2.0**	140.10±2.0**	188.2±1.6**	0.47±0.012**	0.094±0.004***
Silymarin 25mg/kg	66.10±1.2**	134.2±1.4**	84.3±1.65**	0.40±0.01**	0.092±0.02**

Values are mean ± S.E.M, n=6; $^{ns}p>0.05$, *p<0.05, **p<0.01 Vs control.

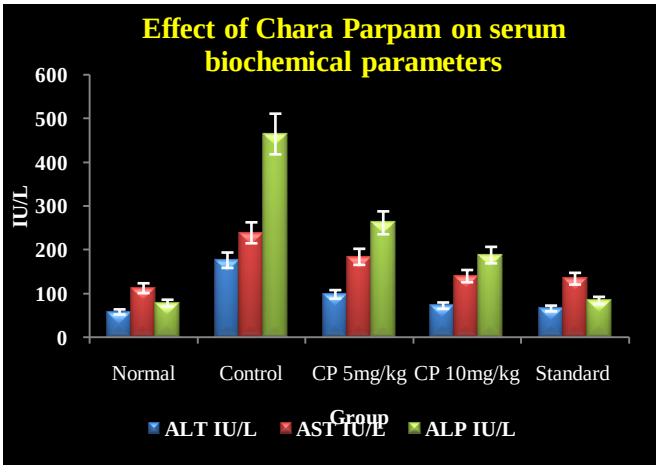


Fig No.2. Showing the effect of *Chara Parpam* on serum biochemical parameters.

Table 3: Effect of *Chara Parpam* on serum parameters Total protein, Albumin and ions

GROUP	Total protein g/dl	Albumin g/dl	Sodium mEq/L	Potassium mEq/L
Normal Saline	5.1±0.10	4.2± 0.11	140.12± 0.7	4.85± 0.26
Positive Control	2.2±0.12	2.4± 0.05	75.41± 0.9	2.00± 0.08
Chara Parpam 5mg/kg	3.32± 0.2*	2.55±0.17	84.23± 2.0*	2.81±0.17*
Chara Parpam 10mg/kg	4.21± 0.20**	3.62±0.18**	120.02± 1.4**	4.33±0.16**
Silymarin 25mg/kg	5.18±0.07**	4.46±0.05**	136.13±1.8**	4.62±0.14**

*Values are mean ± S.E.M, n=6; $^{ns}p>0.05$, *p<0.05, **p<0.01, Vs positive control.*

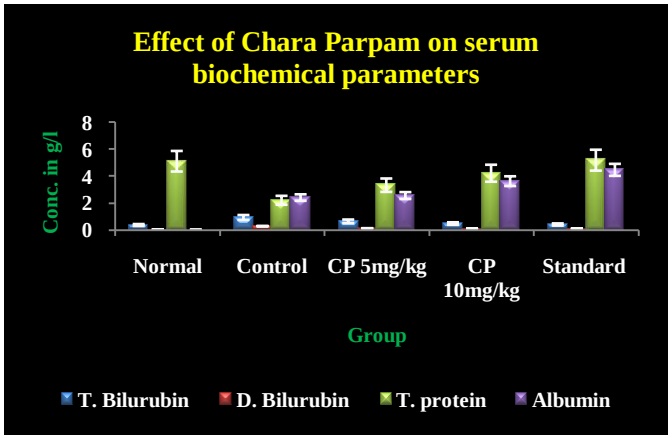


Fig No.3. Showing the effect of *Chara Parpam* on serum biochemical parameters

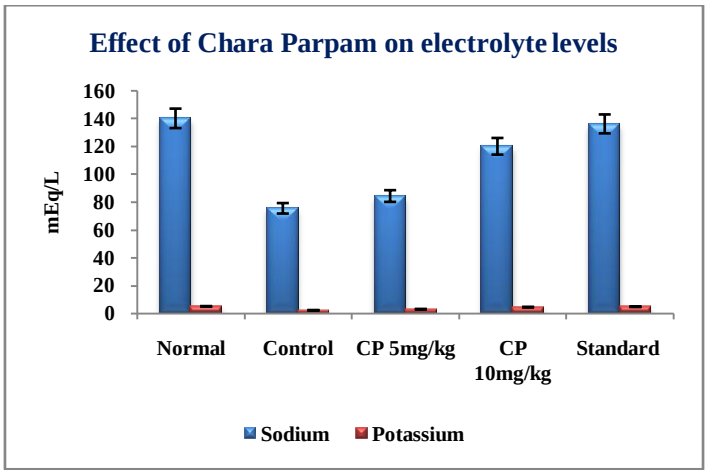
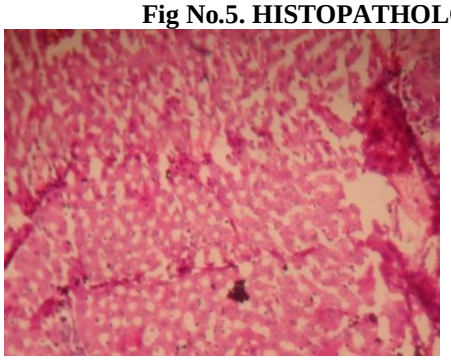
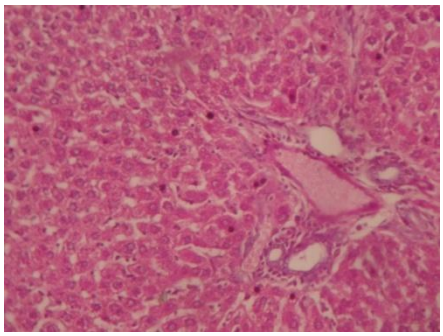


Fig No.4. Showing the effect of *Chara Parpam* on electrolytes level

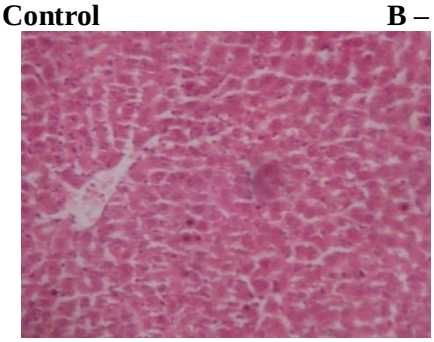
Fig No.5. HISTOPATHOLOGICAL STUDIES OF RAT LIVER



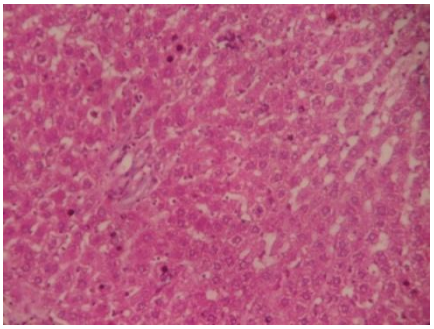
A – Toxicant Control



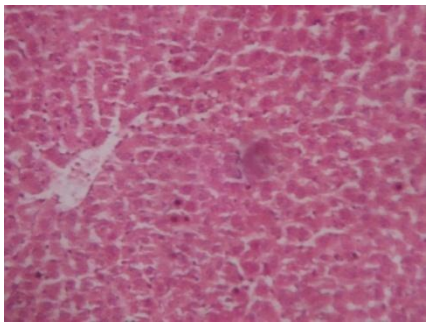
B – Normal group



C - Standard



D – *Chara Parpam* 5 mg



E – *Chara Parpam* 10 mg

V. Conclusion

The results obtained from estimation of biochemical parameters suggesting that, *Chara Parpam* at the dose 10 mg/kg possess significant hepatoprotective property in CCL_4 induced liver toxicity in rat model. This study indicated the dose-effect relationship of *Chara Parpam*. The histopathological studies supported the results of biochemical tests treated with *Chara Parpam* 10mg/kg showing less damage in the cyto architecture of the liver.

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