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HEPATOPROTECTIVE ACTIVITY OF *SEENTHIL CHOORANAM*

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ABSTRACT
Siddha medicine is one of the most ancient medical systems of South Asian region of the world. *Seenthil chooranam* is one of the Siddha medicinal drug with huge medicinal properties. The drug *Seenthil chooranam* mentioned in classical siddha text *Agasthiyar paripuranam* – 400 for its hepato-protective activity. Liver is a vital organ which participates in biotransformation, detoxification of substances and excretion of toxins and chemicals. But there are some instances where the liver hepatocytes undergo hepatotoxicity. Hepato protective effects are attributed to the efficient antioxidant activity of the *Seenthil Chooranam*, which restores the peroxidases activity in the liver. Therefore, *Seenthil Choornam* by regulating these substances carry out hepato-protective activity. In this research work activity of *Seenthil choornam* on SGPT, SGOT and SALP, Total bilirubin and Total protein on CCl4 induced liver damage in rats. Healthy and either sex of five groups of wistar albino rats of (150- 200g) body weight were used for this study purpose. Group I received 10 ml/kg body weight of ghee orally once daily for 9 days. Groups III-IV rats were pre-treated with the extract of Seenthil Choornam 200 and 400 mg/kg for 7 days once daily by gastric intubation. Groups II - V rats were administered 1.25 ml/kg of CCl4. Group V were fed with standard drug Silymarin 25mg/kg. In this study blood samples revealed increased levels of serum enzymes SGOT, SGPT and SALP were detected in mouse treated with CCl4. And administration of S.Chooranam decreased the hepatocyte damage same like the standard drug given.

KEYWORDS: Hepato-protective, *Seenthil chooranam*, liver, Enzymes.

INTRODUCTION
Indigenous medicines such as Ayurveda and Siddha system of medicine play an important role in the South Asian region of the world. They are the medications prepared in the traditional way of medicine preparation.[1] And mainly from the herbal products which has no side effects compared to the allopathy medicines. There are a variety of Siddha drugs available to cure the majority of diseases around the world.[2]

Seenthil chooranam is one of the Siddha medicinal drug which has a wide range of medicinal properties. Mainly there are 19 types of medicinal actions of the *Seenthil chooranam* such as antipyretic, adaptogen, anti-constipation, antacid, anti-gout, anti-cancer, antipruritic, anti-inflammatory, carminative, antioxidant, anti-stress, digestive stimulant, hematognic, febrifuge, immunomodulatory, gastrointestinal protective, mild analgesic and rejuvenate.[3]

Liver is a vital organ which takes part in the human body system. Which participates in biotransformation, detoxification of substances and excretion of toxins and chemicals. But many factors can lead to hepatotoxicity of the liver which includes some organic solvents, drugs, alcohol and also malfunctioning of many enzymes.[4,5] Due to this imbalance there will be damage in hepatocytes or liver which is called hepatitis. This hepatitis can also occur due to infections. The literature highlights evidences that the *Seenthil chooranam* has the power of protecting the liver from hepatitis conditions.[6]

This *Seenthil chooranam* is used to treat indigestion, flatulence, heartburn, jaundice and splenomegaly. This *Seenthil chooranam* contains *Tinospora cordifolia*,. In the Siddha system of medicine this *Seenthil chooranam* is prescribed for the patients in the dosage for children, 250mg to 1.5 g and for adults 1-3 g is given for 2-3 times a day after meal with ghee or warm water as the adjuvant. It is also considered safe for children, adult and also for pregnant woman.[7,8]

The drug Seenthil chooranam mentioned in classical siddha text Agasthiyar paripuranam – 400, has been used for *Megam* (Diabetic mellitus), *Eelai* (Tuberculosis), *Kasam* (Cough), *Elaipu* (Bronchial asthma), *Eranda vayu* (Scrotal swelling). The ingredients of this formulation are *Seenthil (Tinospora cordifolia)* - 10

palam (350gm) *Karisalai Eclipta Alba)* - 10 palam (350gm) Earthworm (*Eudrilus eugeniae*) - 3 palam (105mg).[9] This *churnam* is a potent hepato protective medicine used to treat inflammations of the liver due to various reasons. Since the main ingredient *seenthil* or *Tinospora cordifolia* has the pharmacological activity of anti-inflammation. This has the property of healing ad modulating the inflammations in the liver and protects the liver hepatocytes from further pathway to cirrhosis.[10] Also *Eclipta alba* another ingredient of this medicine plays an important role elucidated that *Eclipta alba* has Significant hepato-protective activity against paracetmol induced rat.[11, 12] Various solvent extracts of an earthworm, *Eudrilus eugeniae* were reported for anti-inflammatory activity.[13] Researcher was chosen this trial medicine to elucidate synergistic activity of this valuable medicine to protect the liver from the ailments without any side effect.[14, 15]

MATERIALS AND METHODS

Selection of animals

Healthy and either sex of Wistar albino rats of (150-200g) body weight were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: KKCP/2015/034.

The animals were kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the study period. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental design

The wistar rats were randomized into 5 groups comprising 6 animals in each group weighing between 150-200g. Rats in Group I received 10 ml/kg body weight of ghee orally once daily for 9 days. Groups III-IV rats were pre-treated with the extract of *Seenthil Chooranam* 200 and 400 mg/kg for 7 days once daily by gastric intubation. Hepatic damage was induced in Groups II - V rats as described by.[16,17] administration of CCl4 intra peritoneally at the dose of 1.25 ml/kg CCl4 in olive oil (at the ratio of 1:1), 30 min post-dose of *Seenthil Chooranam* on days 8 and 9 as described by.[18] Group V were fed with standard drug Silymarin 25mg/kg; p.o daily for seven days. The animals were fasted overnight and sacrificed on day 10 by cervical dislocation after collection of blood samples.

Blood Sample Collection and Analysis

Blood samples for haematological analysis were collected from all the rats through the retro-orbital venous plexus under ether-induced anaesthesia, into heparinized tubes while the sample for serum biochemistry was collected into plain tubes. From the blood samples collected, packed cell volume (PCV) was determined by micro haematocrit method, haemoglobin concentration (Hb) by cyanmethaemoglobin method while the red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of PCV, Hb and RBC count.[19] Erythrocyte osmotic fragility was determined according to the method by diluting 0.02ml of blood in test tubes containing 0 – 0.9% NaCl in phosphate buffer at pH of 7.4. The tubes were gently mixed and incubated at room temperature (29ºC) for 30 minutes, then centrifuged at 3500rev/min for 10 minutes.[20] The supernatant were decanted and the optical density determined at 540nm using SM22PC Spectrophotometer. Haemolysis in each tube was expressed as a percentage, taking the tube with the highest haemolysis (i.e. Distilled water with 0.0% NaCl) as 100%.

Serum Biochemistry

Whole blood was separated with high speed macro-centrifuge at 3,500 rpm for 10 minutes and serum was separated by Pasteur pipette for analysis of the following biochemical assays; Alkaline phosphatase (ALP) as described by[21], aspartate aminotransferase (AST)[22], alanine aminotransferase (ALT)[23] albumin and total protein.[24]

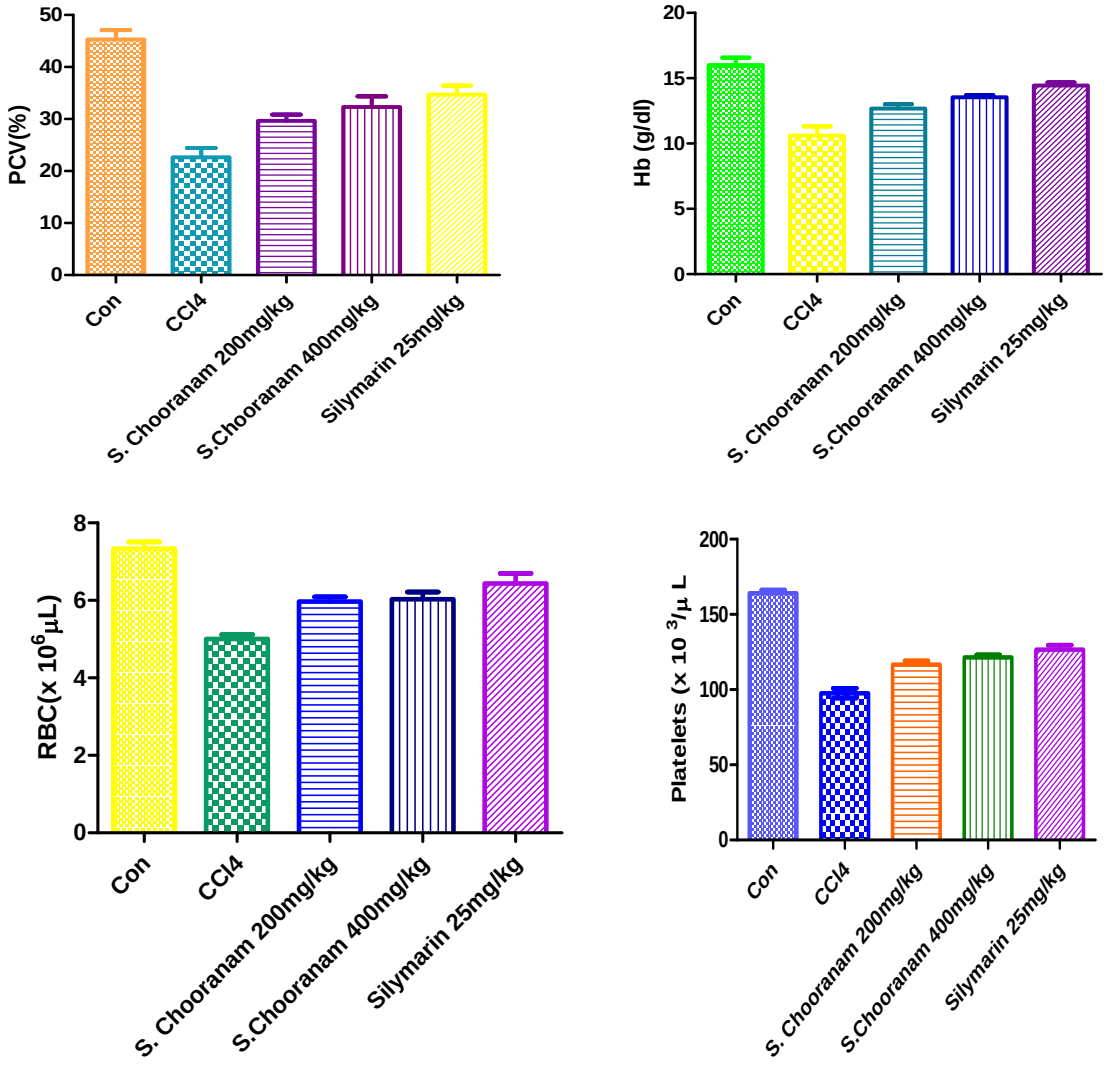
All results were reported as mean ± SEM. They were further analyzed using one way analysis of varients (ANOVA) followed by Tukey's multiple comparison test.

RESULTS

Table 1: Effect of Seenthil Chooranam and Silymarin on haematological parameters of CCl4 induced liver damage in rats.

Parameters	Control	CCl4	CCl4+ S. Chooranam 200 mg/kg	CCl4+ S. Chooranam 400 mg/kg	CCl4+ Silymarin 25 mg/kg
PCV (%)	45.33± 1.76	22.67±1.76 ###	29.67±1.20	32.33±2.02 *	34.67±1.76 **
Hb (g/dl)	16.00±0.57	10.60±0.70###	12.67±0.33	13.53±0.17 **	14.43±0.23 ***
RBC (x 10^6/µ L)	7.33±0.17	5.00±0.11 ###	5.96±0.12*	6.033±0.18 *	6.433±0.26 **
Platelets (x 10^3/µ L)	164.0±2.30	97.67±3.18 ###	116.7±2.40 **	121.3±1.76 **	126.7±2.90 ***
MCV	63.67±0.88	41.33±3.52 ###	47.00±2.08	51.33±1.20 *	54.00±2.00 *
MCH	24.33±1.85	14.33±1.20 #	13.67±0.88	15.33±1.76	16.67±2.40
MCHC	35.00±1.73	45.00±1.73 #	41.67±0.81	40.67±2.18	36.67±2.40
WBC X 10^3	6.93±0.59	9.86±0.17***	9.10±0.17	8.16±0.14 #	7.23±0.20 ###

Values are Mean ± SEM; n = 6 animals in each group: $^{\#}$P<0.05, $\neq^{\#}$ P< 0.01, $^{\#\#\#}$ P<0.001 is considered significant when compared with group I; *P<0.05, **P< 0.01, ***P<0.001 is considered significant when compared with group II by Tukey multiple comparison test.



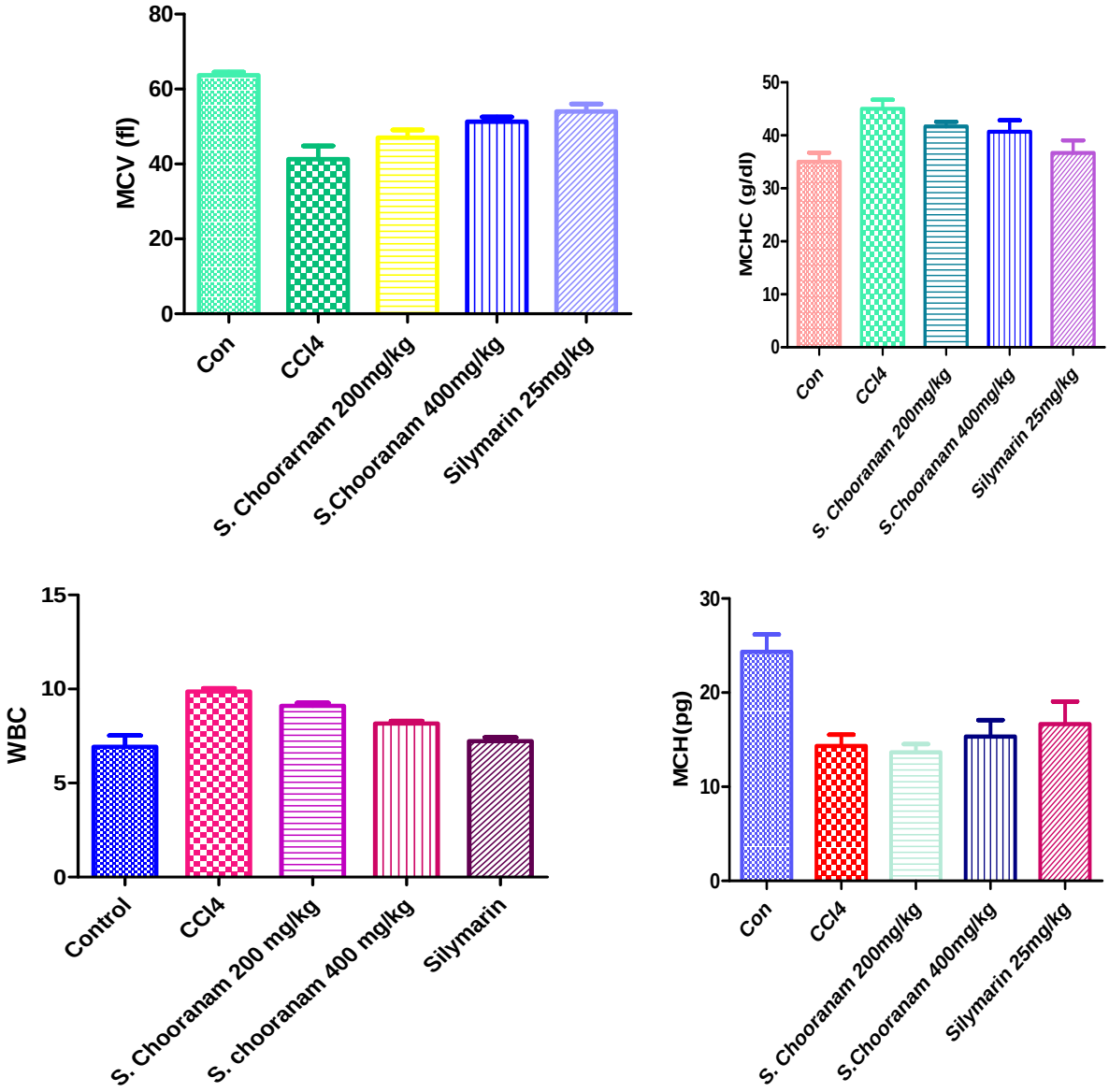
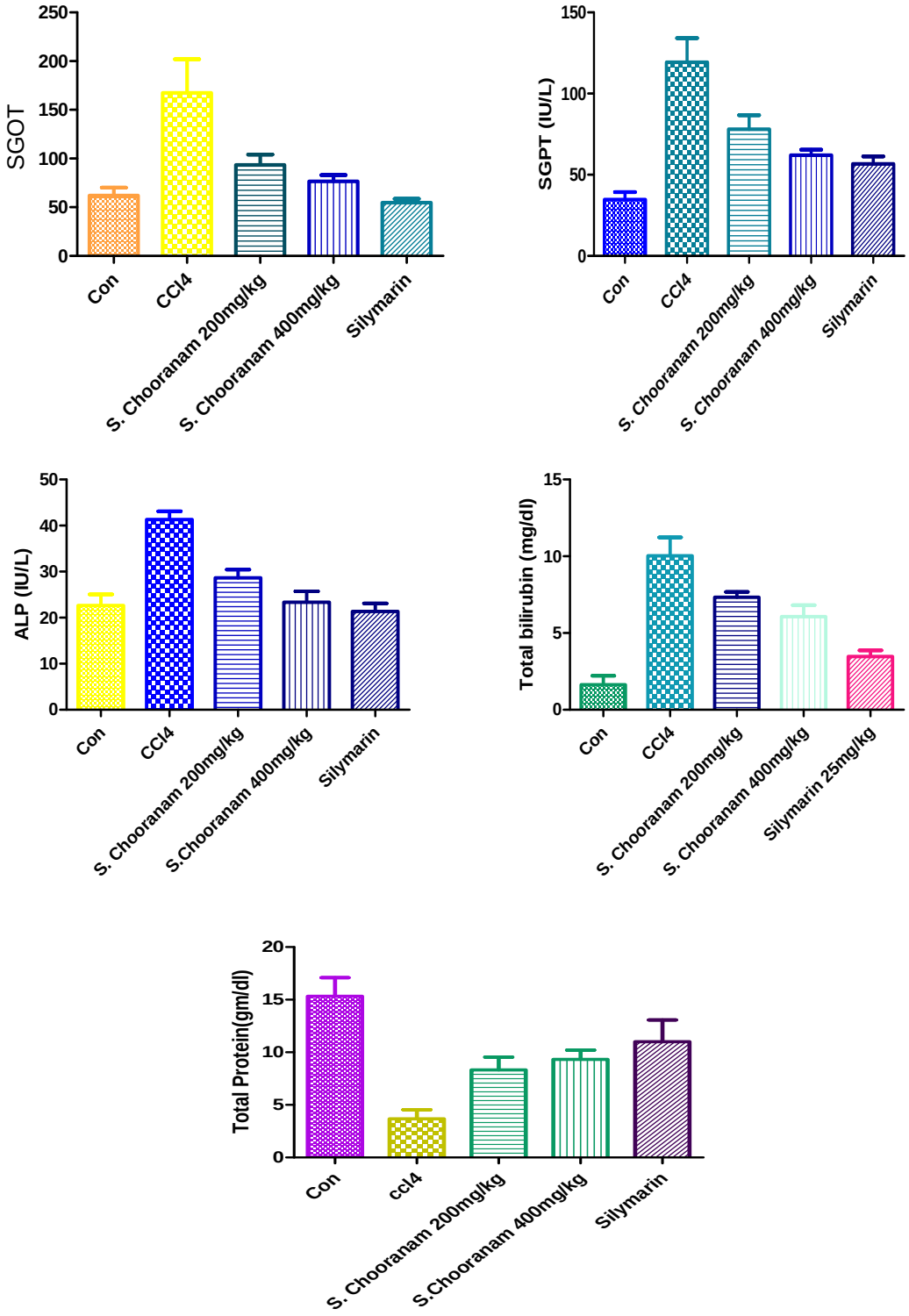


Table 2: Effect of Seenthil Chooranam and Silymarin on serum enzymes (SGPT, SGOT and SALP), Total bilirubin and Total protein on CCl4 induced liver damage in rats.

Group and Treatment Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total Bilirubin (mg/dl)	TOTAL PROTEIN (mg/dl)
Ghee 10ml/kg	62.00±8.08	34.67±4.66	22.67±2.40	1.63±0.58	15.33±1.76
CCl4 Rats 1.25ml/kg (ip)	167.3±34.65##	119.3±14.89 ###	41.33±1.76 ###	10.03±1.20 ###	3.66±0.88##
Seenthil chooranam 200mg/kg+ CCl4 1.25ml/kg (ip)	93.33±10.73	78.00±8.71*	28.67±1.76 **	7.33±0.35	7.66±1.20
Seenthil chooranam 400mg/kg+ CCl4 1.25ml/kg (ip)	76.67±6.36*	62.00±3.46 **	23.33±2.40 ***	6.06±0.75*	9.33±0.88
Silymarin 25 mg/kg 25 mg/kg+ CCl4 1.25ml/kg (ip)	54.67±4.05 **	56.67±4.66**	21.33±1.76 ***	3.46±0.40 ***	11.00±2.08 *

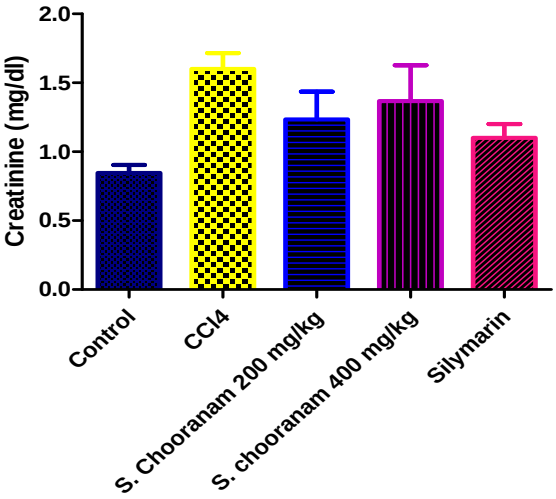
Values are Mean ± SEM; n = 6 animals in each group: $^{\#}$P<0.05, $^{\#\#}$ P< 0.01, $^{\#\#\#}$ P<0.001 is considered significant when compared with group I; *P<0.05, **P< 0.01, ***P<0.001 is considered significant when compared with group II by Tukey multiple comparison test.

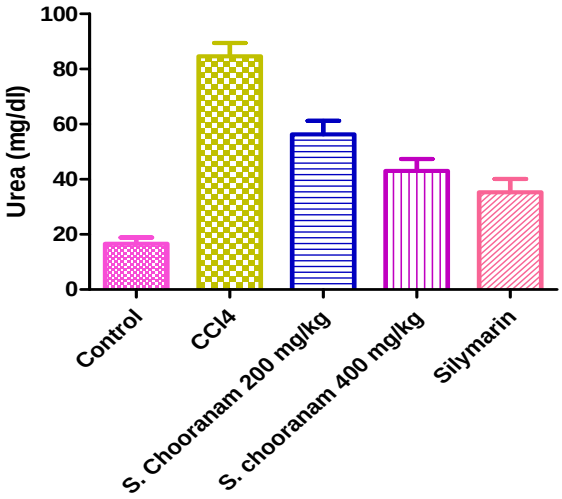


Effect of Seenthil Chooranam and Silymarin on serum Creatinine and Urea, on CCl4 induced liver damage in rats

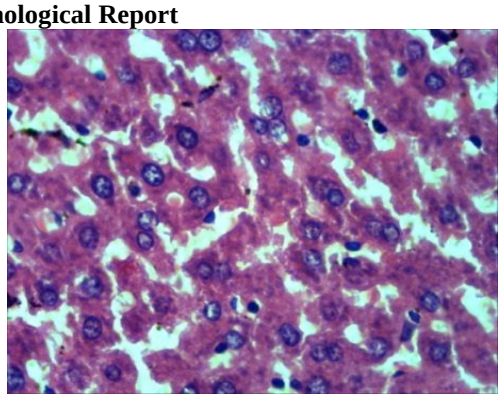
Parameters	Control	CCl4	S. Chooranam 200 mg/kg	S. chooranam 400 mg/kg	Silymarin
Creatinine (mg/dl)	0.84±0.05	1.60±0.11	1.23±0.20	1.36±0.26	1.10±0.10
Urea (mg/dl)	16.67±2.18	84.67± 4.80 ***	56.33±4.91 ##	43.00±4.35 ###	35.33±4.80 ###

Values are mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 compared to control rats.### P<0.001, ##P<0.01, #P<0.05 compared with group II by Tukey multiple comparison test.

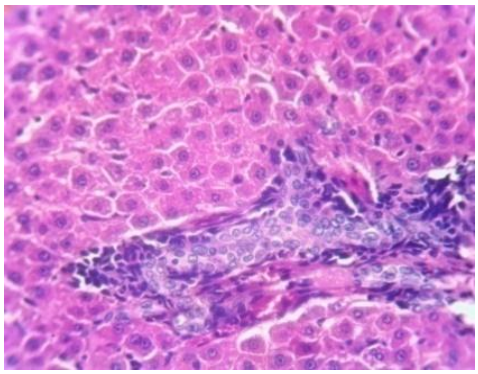




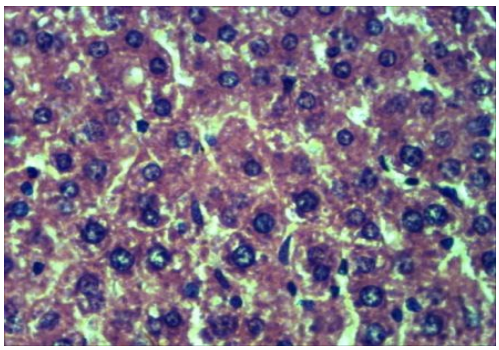
Histopathological Report



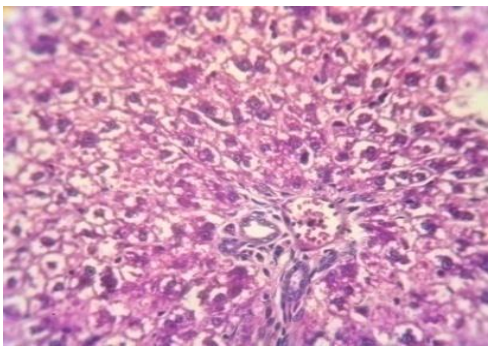
Group 1: Control.



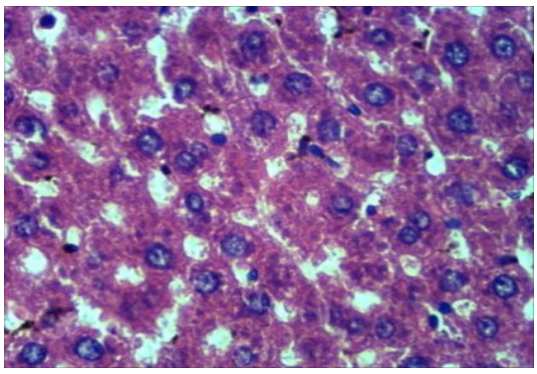
Group 2: CCl4treated.



Group 3: S. Chooranam.



Group 4: S.Chooranam 400mg/kg+CCl4 200mg/kg + CCl4.



Group 5: Silymarin 25mg + CCl4.

- Group1: Photomicrograph of liver tissue of control rats showing normal hepatic cells with central vein (CV) and sinusoidal dilation (S).
- Group 2: Photomicrograph of liver tissue of rats treated with CCl4 showing severe Centrilobular necrosis (N) with disappearance of nuclei.
- Group 3: Photomicrograph of liver tissue of rats treated with S. Chooranam at 200mg/kg showing mild degree of necrosis (N) with mild inflammatory cells.
- Group 4: Photomicrograph of liver tissue of rats treated with S. Chooranam at 400mg/kg showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area (M).
- Group 5: Photomicrograph of liver tissue treated with Silymarin at 25mg/kg showing normal hepatocytes, portal vein (V) and portal artery.

DISCUSSION

The serum marker enzymes, SGOT, SGPT and SALP more specific index of liver cell damage and oxidative stress, which stimulate the release of amino transferases from hepatocytes into the blood.[25,26]

This study reveals that increase in the activity of the serum enzymes SGOT, SGPT and ALP were detected in mouse treated with CCl_4 (Group II). However, the activities of these serum enzymes were significantly ($P < 0.001$) lower in treated with *S.Chooranam* (Group 3 and 4) than in Group 2.

This present study confirmed in both the doses of *S.Chooranam* treatment (200 and 400 mg/kg body wt.) significantly improved the effect of CCl_4 induced liver damage.

The Histopathological studies showed that CCl_4 administered rat caused pathological changes in liver including severe centrilobular necrosis with disappearance of nuclei (Group 2). The liver with mild change in showing mild degree of necrosis with mild inflammatory cells of rats treated with *S.Chooranam* 200mg/kg and CCl_4 (Group 3), the liver was almost has normal appearance of rats treated with *S. Chooranam* at 400mg/kg and CCl_4 (Group 4), showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area Indicating that the administration of *S.Chooranam* decreased the hepatocyte damage and silymarin also has the same effect (Group 5). Control rats showed the normal appearance of liver without any histological alterations (Group 1).

CONCLUSION

In the present study the above parameters were analyzed and concluded that *S.Chooranam* has significant produced hepatoprotective activity against CCl_4 induced rat.

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