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Study of hepatoprotective activity of Liv' Well liver tonic in CCl_4 induced hepatotoxicity in rats

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ABSTRACT

The hepatoprotective activity of Liv'Well liver tonic was investigated using CCl_4 (1ml/kg i.p for 7 days from 3rd to 10th day) albino Wistar rat liver. The Liv'Well liver tonic was administered orally at a dose of 200 and 400mg/kg po, for 10 consecutive days. The doses were determined from the acute oral toxicity study in albino mice. The CCl4 was administered to all the groups except group I (normal control). Liv-52 syrup 5ml/kg, p.o was used as a standard hepatoprotective drug. After 24h of the last day treatment, blood was withdrawn by retro orbital puncture and serum SGOT, SGPT, ALP, total bilirubin was determined. Then the animals were sacrificed under ketamine over anesthesia (150mg/kg ip) and the liver was isolated and processed for the histopathological investigation. The rats treated with Liv'Well + CCl_4 significantly reduced the levels of SGOT, SGPT, ALP, total bilirubin when compared to CCl_4 hepatotoxic rats. Histopathological examination also reveals the hepatoprotective activity of Liv'Well liver tonic. From the results of the present study, it is concluded that Liv'Well- liver tonic formulated by M/s Suguna Foods Pvt Ltd, Herbal Division, Suguna Lifeherbs Coimbatore, TN, possesses superior hepatoprotective properties that may serve to protect users against diseases associated with hepatic diseases

Keywords: Liv'Well, liver tonic, Liv-52 syryp, total bilirubin, ketamine.

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INTRODUCTION

Liver is the vital organ, which is maintaining of metabolic reactions in the human body. But unnecessary food habits, consuming of impure drinks may bring problem in functioning of liver. Consuming more number of drugs also causes liver damage, intake of alcohols, junk food etc are also questioning the functional ability of the liver by damaging the liver architecture [1]. There is no rational therapy available for liver disorder, and it's still challenge to modern science. Hepatic injury can be life threaten when entirely or most of the liver exposed to hepatotoxin .In elucidating the mechanism of the liver damage therefore , halogenated alkanes such as carbon tetrachloride are widely used as model compound to induce hepatotoxicity, it is one of the common ailments resulting in serious debilities ranging from metabolic disorders to even mortality . In the recent years , importance is being given to Ayurvedic poly herbal formulations due to their effective therapeutic action and lack of side effects. Damage of liver can be assessed by the elevated levels of serum enzymes like SGOT, SGPT and bilirubin. [2]

Carbon tetrachloride (CCl_4):- Carbon tetrachloride is widely used chemical to induce liver damage. The reactive free radicals that can bind covalently to cellular macromolecules forming nucleic acid, protein and lipid adducts through the induction of hypo methylated ribosomal RNA that inhibits the protein synthesis and finally can affect hepatocellular calcium homeostasis. It results in inflammation, centrilobular, steatosis, apoptosis necrosis, if the damage exceeds the repair capacity it causes fibrosis and cirrhosis. [3]

Paracetamol:

In cases of paracetamol overdose, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the cytochrome P450 system to produce NAPQI. As a result, hepatocellular supplies of glutathione become depleted, as the demand for glutathione is higher than its regeneration. NAPQI therefore remains in its toxic form in the liver and reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and death, leading to acute hepatic necrosis. In animal studies, hepatic glutathione must be depleted to less than 70% of normal levels before hepatotoxicity occurs.

Alcohol

Alcohol can change in-vivo membrane lipid composition and fluidity, it affect cellular function. Over consumption of alcohol causes fatty infiltration hepatitis and cirrhosis .Due to increase in hepatic lipid peroxidation leads to alteration in membrane phospholipids composition, it results in the enhanced generation of oxy free radicals during oxidation in liver, which elevates the level

of glutamyl trans peptidase membrane bound enzymes in serum, it decrease activity of super oxidase dismutase and catalase, it increases the activity of glutathione in liver. [4]

There is potent indigenous poly herbal formulations available for the treatment of liver disorders in different parts of the world and most of them have not yet scientifically been validated. If they are conducted, it could lead to the development of cost effective drugs. Natural remedies from herbal medicinal preparations are recommended for the treatment of liver disorders.[5]

Liv' Well is a liver tonic developed by M/S Suguna Foods Pvt. Ltd., Herbal Division, Suguna Lifeherbs, Coimbatore, Tamil Nadu, India. But there is no scientific study for its hepatoprotective activity. Hence the present study was undertaken to explore the key behind the use of Liv' Well as a liver tonic against various experimentally induced hepatotoxicity animal models.

MATERIALS AND METHOD

Sample

Liv' Well powder was obtained from M/S Suguna Foods Pvt. Ltd., Herbal Division, Suguna Lifeherbs, Coimbatore, Tamil Nadu, India. The silymarin standard drug was procured from local medical store. All biochemical kits were procured from authorized supplier of Erba diagnostics. All other chemicals and reagents are of analytical grade and purchased from authorized suppliers.

Experimental animals

Female Albino mice (6) weighing between 20-25 g and thirty albino Wistar rats (150-200g) of either sex were procured from Sri Venkateshwara Enterprises Bengaluru. The animals were acclimatized for 7 days under standard husbandry conditions, i.e., room temperature of (26±10) ^{o} C, relative humidity of 45-55% and light: dark cycle of 12:12 h. The experimental protocol was presented in the Institutional Animal Ethics Committee of PES College of Pharmacy (CPCSEA Reg No-66/PO/Ere/S/02/CPCSEA dated 20-3-2015), Bengaluru meeting held on 16-6-2015 and IAEC approval was obtained (PESCP/IAEC/17/2015). The animal experiments were conducted according to the Committee for the Purpose of the Control and Supervision and Experiments on Animals (CPCSEA) guidelines.

Acute oral toxicity study of Liv' Well-liver tonic for the dose measurement

Acute oral toxicity study was performed according to the fixed dose method of OECD Guide line No. 425. Female Albino mice (6) weighing between 20-25 g were used for the acute oral toxicity study. The animals were kept fasted overnight prior to acute experimental procedures. The dose of 50, 100, 300, 1000, 2000 and 3000mg/kg body weight was given to 6 different animals &

observed individually once during first 30 minutes and periodically during the first 24h with special attention given during the first 4 h and daily thereafter for a total of 14days additional observations like change in skin and fur, eyes and mucous membranes and also respiration, circulation, autonomic and CNS and somato motor activity and behavior pattern was observed. [3]

Study design of hepatoprotective activity-

Group - I Normal saline for 10 days. Group - II CCl_4 1ml/kg (i.p) for 7 days (from 3rd to 10th day). Group- III CCl_4 1ml /kg for 7 days, and reference standard Liv-52 syrup 5ml/kg, p.o for10 days. Group- IV, V Administered with 200mg/kg (p.o) and 400mg/kg (p.o)for 10 days with CCl_4 1ml/kg for 7 days.[4]

24 h after the last day (10th day) administered, animals were anaesthetized by using ether anesthesia. The animal was scruffed with thumb and forefinger and the skin around the eye is pulled taut. A capillary tube was inserted into the medial canthus under the nictitating membrane of the eye. Slight thumb pressure with rotation motion was applied to puncture the tissue and to enter the capillary tube tip into the plexus/sinus. As soon as the sinus was punctured, blood entered the tubing by capillary action. When the desired amount of blood was collected, the tube was withdrawn and a clean gauze pad was applied on the eye to ensure hemostasis. The collected blood was allowed to clot and centrifuged at 2500 rpm for 15 min and serum separated and transferred to clean eppendorff tubes and used for the estimation of SGOT, SGPT, ALP, direct bilirubin and total bilirubin biochemical marker enzymes. Then the animals were sacrificed using ketamine overdose anesthesia. The liver was isolated and used for the histopathological study.

SGOT

(Serum glutamic oxalo acetic transaminase) or AST (Aspartate transaminase) level was measured using Erba diagnostic kit and semi autoanalyzer. Under standard conditions the absorbance was measured at 340 nm and the serum SGPT activity was measured using the formula (IU/L) = ΔA/min X Factor (1768). The normal serum SGOT levels in rats 45.7 to 80.8U/L

SGPT(Serum glutamic pyruvic transaminase) or ALT (Alanine transaminase)

Serum SGPT level was measured using Erba diagnostic kit and semi autoanalyzer. Under standard conditions the absorbance was measured at 340 nm and the SGPT activity was calculated using the formula SGPT activity (IU/L) = ΔA/min X Factor (1746). The normal serum SGPT levels in rats 17.5 to 30.2U/L.

ALP

Serum Alkaline Phosphatase (ALP) level was measured using Erba diagnostic kit and semi autoanalyzer. Under standard conditions the absorbance was measured at 405 nm and the ALP activity was calculated using the formula ALP activity (IU/L) = ΔA/min X Factor (2764). The normal serum ALP level in rats 56.80 to120 U/L).

RESULTS AND DISCUSSION

Table -1 Effect of Liv'Well liver tonic on SGOT, SGPT, ALP, and total bilirubin.

GP n=6	SGOT IU/L	SGPT IU/L	ALP IU/L	Total bilirubin mg/dL
I	75.01±1.33	79.5±0.99	179±8.18	1.67±0.39
II	183±3.89****	173.2±2.02****	443±49.17****	48.60±1.26****
III	76.1±1.54****	88.51±5.30****	217.5±62.04*	3.18±0.33****
IV	168.8±3.79**	124.0±15..55***	194.8±23.2***	19.93±9.44****
V	80.77±2.20****	95.6±5.84****	279.6±27.45**	4.38±0.6****

Each value is expressed as mean ±SEM (n=6) animals in each group. The second group positive control was compared to the first group by non –paired *t* test followed by <p 0.001 for SGPT, <p0.001 for SGOT ,ALP for <p 0.004,Direct bilirubin for <p0.001,Total bilirubin for <p0.001 as compared to the second group with remaining groups by one way ANOVA followed by Dunnett' s multiple comparisons test .

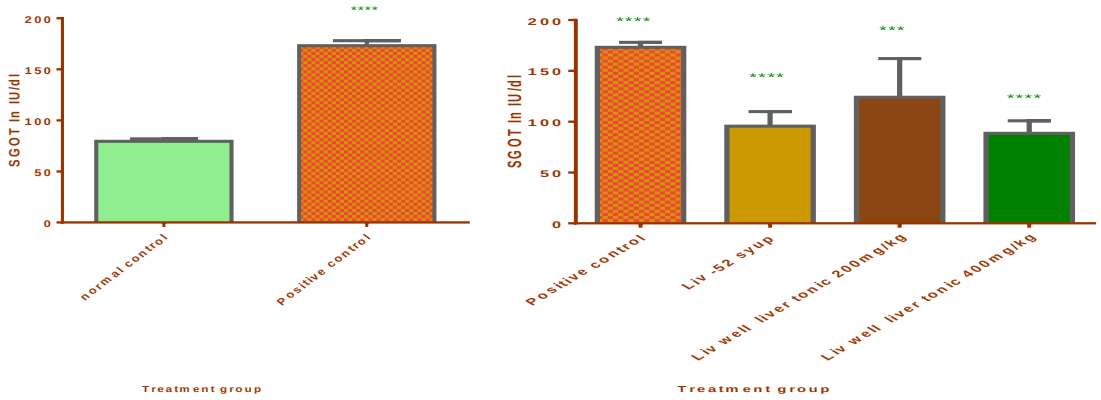

Figure 1: Effect of Liv'Well on SGOT

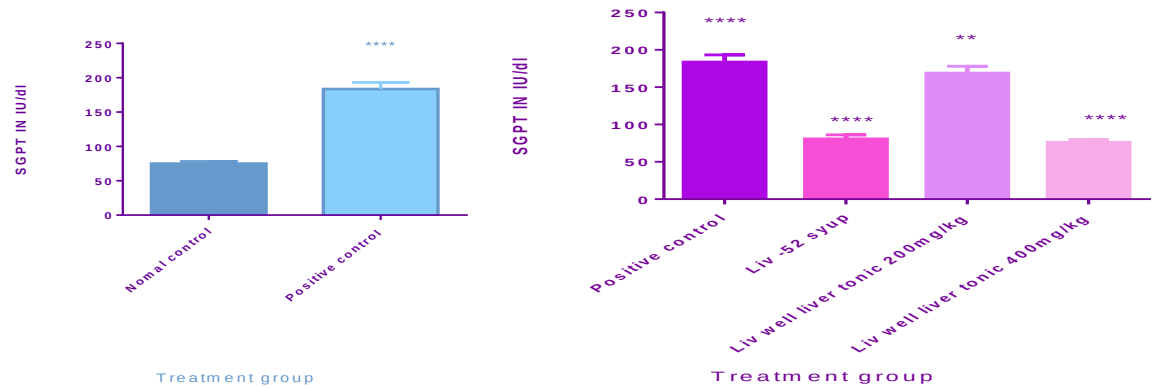

Figure 2: Effect of Liv'Well on SGPT

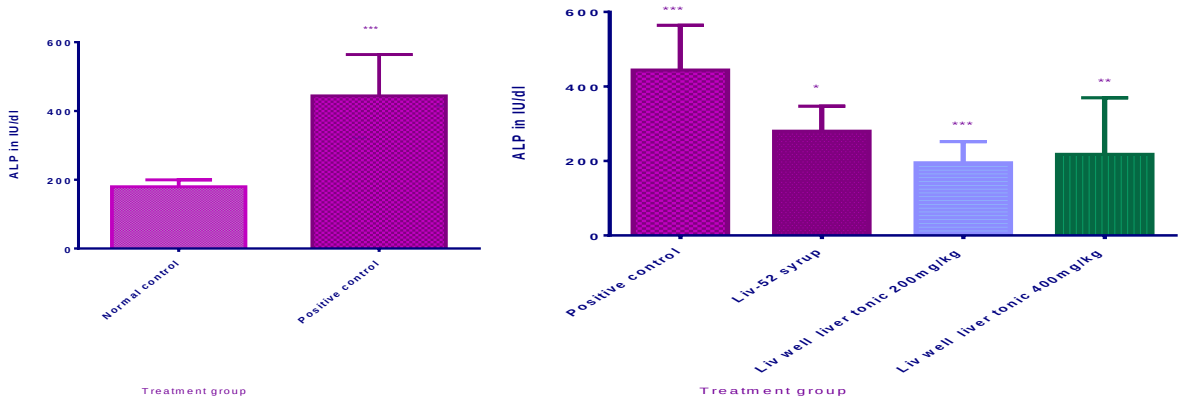

Figure 3: Effect of Liv'Well on serum ALP

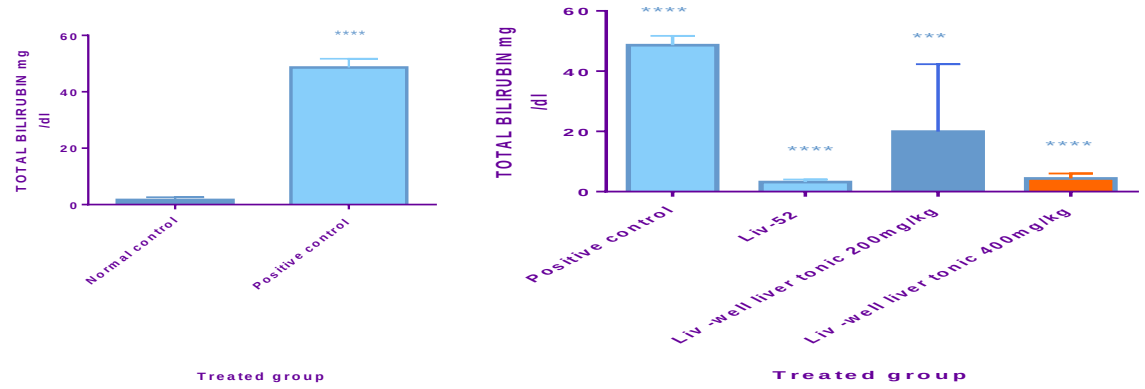

Figure 4: Effect of Liv'Well on total bilirubin

Histopathology study of the liver- The liver was collected in 10% formalin, and section of liver tissue was embedded with paraffin wax, stained with heamotoxylin and eosin and carried out for microscopical examination. The architecture of the each rat liver was analyzed.

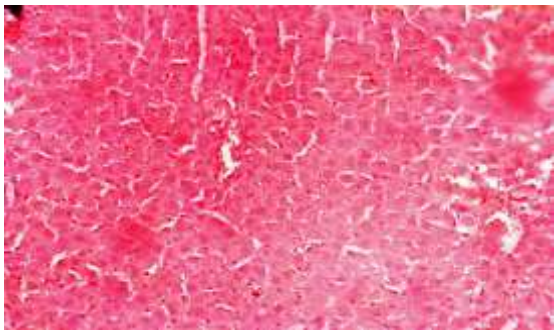

Figure 5 (Normal)

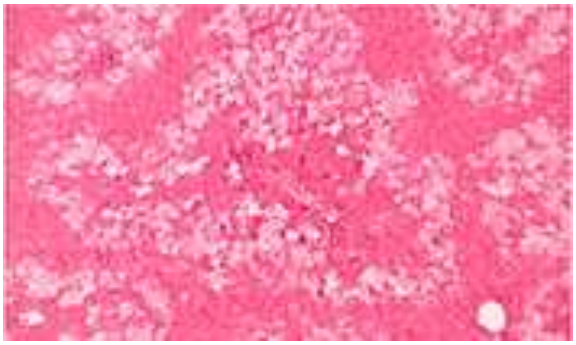

Figure 6 (CCl_4)

Figure 5 Normal lobule architecture is seen. Hepatocytes and their nuclei are well visible.

Figure 6 Extensively centrizonal necrosis is seen .Hepatocytes with nuclei are discrenible ,fatty changes,portal inflammation are seen.

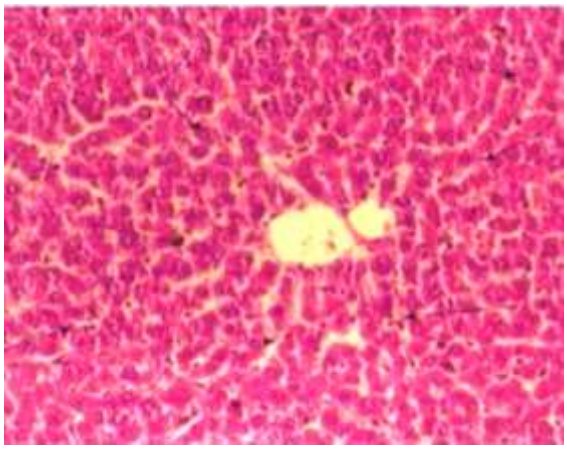

Figure 7 (Liv-52)

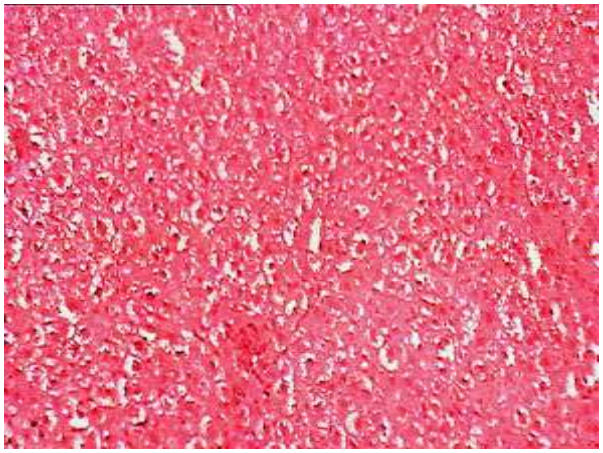

Figure 8 (Liv'Well 200mg/kg)

Figure 7 Mild hepatocytes swelling are indicated by constricted sinusoids. Inflammatory cells are seen mostly around central veins. Necrosis is absent.

Figure 8 Remarkable feather degeneration of hepatocytes is seen around the central vein, fatty changes are seen .Necrosis are not seen, fibrosis is absent.

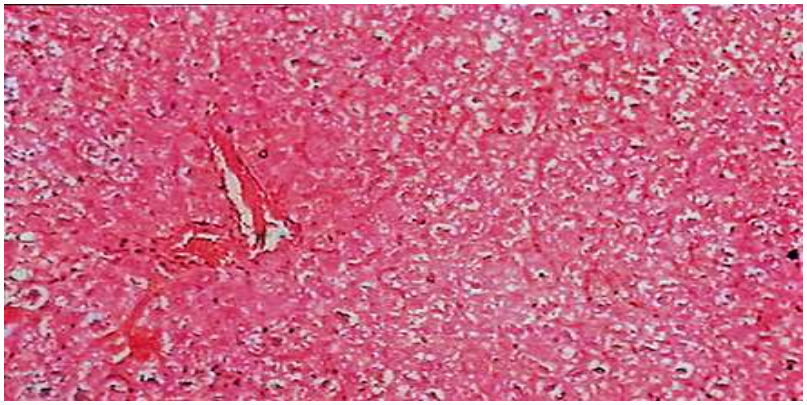

Figure 9 (Liv'Well 400mg/kg)

Figure 9 Remarkable feather degenearartion of hepatocytes is seen around the central vein ,fatty changes are seen ,necrosis are not seen , fibrosis absent.

The Liv' Well -liver tonic was found to be nontoxic up to the dose of 2g/kg wt and there was no behavioral changes or death of tested animals. The phytochemical screening indicates the presence of flavonoids, saponins. CCl_4 has been extensively studied as a liver toxicant, and it metabolites such as trichloromethyl radical and trichloromethylperoxy radical are reported to be involved in pathogensis of liver. The biomarker serum enzymes SGOT, SGPT, ALP, total bilirubin have significantly reduced elevated level in the Liv' Well -liver tonic treated group as compared to the CCl_4 group. Result of present study was given in (Table 1). The histopathological study reveals that in the rat liver of normal group normal architecture is seen, the hepatocytes and their nuclei are well visible. But in the rat of group II (CCl_4 induced liver injury) extensively centrizonal necrosis is seen. The CCl_4 and std Liv 52 administered rat liver histology shows the hepatocytes with nuclei are discrenible, fatty changes, portal inflammation are seen. Mild hepatocytes swelling are indicated by constricted sinusoids. Inflammatory cells are seen mostly around the central veins. Necrosis is absent. The rats liver histopathology (treated with CCl_4 and 200mg/kg p.o. Liv' Well liver tonic) shows the remarkable feather degeneration of hepatocytes is seen around the central vein, fatty changes are seen .Necrosis are not seen, fibrosis is absent. The rats liver histopathology (treated with CCl_4 and 400mg/kg p.o. Liv'Well liver tonic) shows the remarkable feather degenearartion of hepatocytes is seen around the central vein ,fatty changes are seen ,necrosis are not seen ,fibrosis absent. Representative photographs of histopathological changes showing effect of test materials on the rats intoxicated with carbon tetrachloride in found the regeneration of the hepatocytes, confirms the hepatoprotective activity

CONCULSION

In the present study, the administration of Liv' Well -liver tonic showed protection against the toxic effects of CCl_4. The hepatoprotective effect was further concluded by the histopathological examinations of the liver sections, which reveal that the normal liver shape was damaged by hepatotoxin intoxication. In the liver sections of the rats treated with Liv'Well -liver tonic and intoxicated with CCl_4 the normal cellular shape was retained as compared to Liv -52 syrup, there by confirming the protective effect of the formulation. Liv'Well-liver tonic significantly reduced the levels of SGOT, SGPT, ALP, total bilirubin in Liv'Well + CCl_4 administered rats when compared to CCl4 toxic rats. From the results of the present study, it is concluded that Liv'Well-

liver tonic formulated by M/s Suguna Foods Pvt Ltd, Herbal Division, Suguna Lifeherbs Coimbatore, TN, possesses superior hepatoprotective properties that may serve to protect users against diseases associated with hepatic diseases. The resulting hepatoprotective activity of Liv' well liver tonic could be due to presence of flavonoids and saponins which have hepatoprotective properties.

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