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Evaluation of hepatoprotective activity of *Vediannabedhi chendhooram* in animal models

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ABSTRACT

The Siddha drug Vedi Annabedhi Chendhooram (VABC), basically a proprietary Medicine mentioned in Siddha Literature, is evaluated for Hepatoprotective activity in animal model. To evaluate the efficacy of VediAnnabedhi Chendhooram (VABC) in the management of Hepatocellular damages. The Hepatoprotective activity of the drug was studied in CCl_4 induced hepatic damage in Wistar albino rats of either sex. The rats in prophylactic groups were treated with Vedi Annabedhi Chendhooram with honey at the dose of 23.4 mg and 50 mg / kg for 14 days and standard drug Silymarin at the dose of 100 mg / kg. The results showed that CCl_4 administration significantly damaged the liver and the drug administration significantly reduced the level of liver marker enzymes, increase the total protein content and minimize the Histopathological changes. The observations clearly indicate that the drug Vedi Annabedhi Chendhooram with honey possess Hepatoprotective activity with minimal toxicity and could offer promising role in the management of liver damage caused by CCl_4.

Key words: Hepatoprotective, *Vedi Annabedhi Chendhooram (VABC)*, *Vediuppu*, Carbon TetraChloride.

INTRODUCTION

Siddha system has its own well developed chemistry and *Siddhars* tireless striving in the direction of development has resulted in genesis of thousands of mineral and metallic preparations for the treatment of chronic ailments. In Siddha literature some of the salts and minerals are properly indicated for Liver diseases like *Kaamalai* (Jaundice). *VediAnnabedhi Chendhooram (VABC)* studied in this article is a herbo-mineral compound made by using *Annabedhi* (Ferrous Sulphate) and *Vediuppu* (Potassium nitrate) processed with Lemon Juice in a prescribed manner, it is being used with adjuvant like Honey for the treatment of Liver disorders [1].

In Indian system of Medicine, the raw drug *Annabedhi* is having the actions of alterative, diuretic and is being used as haematinic and employed for the treatment of liver disorders. It is also used for debility following on Malaria, Kala-azar, etc [2]. One of the distillation products made by using raw drugs *Annabedhi* and *Vediuppu* which is recommended for the relief of all liver complaints like cirrhosis of Liver with ascites is doing some good, improved prognosis[3]. (Studied by Dr. Koman).

Lemon juice which was used in the preparation of drug contains many citroflavanoids which act as potent antioxidant and free radical scavengers has been studied and published. Lemon is one of the rejuvenating medicines according to Siddha aspect [4]. There is also a tacit belief that the adjuvant used for a drug would also modify the potency of drug and curative capability synergistically for better therapeutic results. All these aspects drove the author to evaluate the hepatoprotective action of the drug along with the given adjuvant.

Basically *Vedi Annabedhi Chendhooram (VABC)* is a proprietary medicine being used to cure liver disorders such as Jaundice, enlargement of liver and spleen - hepatosplenomegaly and Anemia [5]. Until now, no research has been

reported on the hepatoprotective activity of *Vedi Annabedhi Chendhooram (VABC)*. In view of above, the present study was designed to evaluate the hepatoprotective effect of *Vedi Annabedhi Chendhooram (VABC)* with honey against CCl_4 induced liver damage in rats.

EXPERIMENTAL SECTION

1.1. PREPARATION OF DRUG

The raw drugs were identified and authenticated according to the reference in Siddha Text *Gunapadam* 2nd & 3rd Part.

1.1.1.Purification of *Annabedhi*:

The raw drug *Annabedhi* was powdered well, kept in a mud vessel and fried well until it was completely oxidized. The purified *Annabedhi* is whitish in color and powdery in nature [1].

1.1.2.Purification of *Vediuppu:*

The raw drug *Vediuppu* was powdered well and dissolved in water of four times that of drug and boiled. At the time of boiling lemon juice was added and the waste products floating on the surface of it was removed. Then it was filtered by using cloth and kept undisturbed for one day. The next day water content was filtered off and the salt at the bottom was dried in sunlight. The same process was repeated for Seven times [1]. The purified salt was glossy white in color.

1.1.3.Preparation of drug:

Purified *Annabedhi* – 35 grams and purified *Vediuppu* – 8.75 grams were powdered well and grinded in a mortar with lemon juice for 3 hours. After that it was subjected to *pudam* process i.e, application of heat by using cow dung cakes, three times, until the color of it turns into reddish brown [1]. Then it was powdered well and preserved. It was mixed with honey and stock solution was prepared in such a way that 1 ml solution contains 10 mg of drug.

1.2. BIOCHEMICAL ANALYSIS

The prepared drug *Vediannabedhi Chendhooram* is subjected to quantitative and qualitative analysis in Atomic Absorption Spectrometer (AAS) with air – acetylene, XRD and XRF analysis to determine the metals and minerals in the drug.

The Biochemical analysis of the drug *Vediannabedhi Chendhooram* was done in Mettex laboratories of India, Chennai – 32.

The XRF analysis of the drug *Vediannabedhi Chendhooram* was done in Central Electro Chemical Research Institute, Karaikudi.

The XRD analysis of the drug *Vediannabedhi Chendhooram* was done in Department of Nuclear Physics, University of Madras.

1.3. PHARMACOLOGICAL STUDY

Pharmacological Study of *Vediannabedhi Chendhooram* was carried out in Central Research Institute for Siddha, Arumbakkam, Chennai, after obtaining Institutional Animal Ethical Committee clearance (Proposal No. 16-17/PHARMA/CRIS, 2007).

1.3.1.Animals:

Healthy adult male albino rats (200 – 250 grams) and female albino rats (150 – 200 grams) of Wistar strain were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (Temperature range: 65-75^{o}F and Humidity range: 40-70%). The animals had free access to standard pellet diet (Amrut Laboratory Animal Feed, Nav Maharashtra House, Pune, Maharashtra) and water utilizing aqua guard.

1.3.2.Drugs and chemicals:

The following chemicals were used for the study. Carbon tetrachloride (Merck Specialist Private Ltd., Mumbai), Serum enzyme (SGOT, SGPT, ALP and Bilirubin) and serum total protein, albumin and globulin estimation kit (Bayer Diagnostics Ltd., Mumbai).

1.3.3.ACUTE TOXICITY STUDY

The acute toxicity study was carried out using 'Acute Toxic Class method' as per OECD Guidelines 423. Healthy albino mice of either sex were selected and grouped in to six each (Three males and three females). The animals

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were deprived of diet for four hours and water was given *ad libitum*. The drugs were suspended in honey and administered @ 2000 mg/kg body weight orally. Another group of six animals received vehicle honey only, which served as untreated control. The animals were observed for 24 hrs after administration for mortality and thereafter animals were kept under observation for 14 days.

1.3.3.1. Drug profile:

Route of administration **:** Enteral
Dose level **:** 2000 mg/kg
Human dose of trial drug **:** 130 mg twice a day
Calculation of animal dose **:** 60.0 mg/kg
Frequency of administration **:** Once in a day

The animals were observed for the following gross observations:

Effect on Central Nervous system : a) Stimulation b) Depression
Effect on Respiration : a) Stimulation b) Depression c) Respiratory failure
Effect on Locomotor system : a) Increase in motor activity b) Reduction in motor activity
Effect on skin colour : a) Blanching b) Cyanosis c) Vasodilation
Effect on excretions : a) Salivation b) Lacrimation c) Urination
Other effects like : a) Piloerection b) Tonic and Clonic convulsions c) Opisthotonus d) Ataxia e) Body temperature

1.3.4.Induction of Hepatotoxicity and drug feeding schedule:

2.3.4.1 CCl_4 induced liver damage:

All the animals (30) were weighed and randomly divided into five groups comprising of six rats (3 Male + 3 Female) in each. The experimental protocol for CC14 induced hepatotoxicity is cited in Table 1.

The liver damage was induced by subcutaneous injection of CCl_4 in olive oil (1:1) @ 1 ml/kg once on 13^{th} day in Groups II, III, IV and V. Group I and V were kept as normal (Honey) and toxic control group, respectively. On the other hand, Group II, III and IV were treated with *Vediannabedhi Chendhooram* @ 23.4 mg/kg, *Vediannabedhi Chendhooram* @ 50 mg/kg and *Silymarin* @ 100 mg/kg orally, respectively.

The dose of the drug was calculated on the basis of results from acute toxicity studies ($1/10^{th}$ of the maximum tolerated dose). Blood samples were collected through retro orbital sinus of all the animals 48 hrs after $CC1_4$ administration. The blood samples were estimated for biochemical parameters such as SGOT, SGPT, ALP, Bilirubin, Total protein, Albumin and Globulin.

After blood collection all the animals were weighed and euthanized under ether anesthesia. All rats were subjected for gross lesion on liver and the liver were collected, weighed and preserved in neutral buffered 10% (V/V) formalin for histopathology. These were processed for paraffin embedding using ethyl alcohol as dehydrant and xylene as clearing agent. Paraffin sections of liver, about 4-5 µm thickness, were stained with haematoxylin and eosin. These sections were examined for histopathological changes and the cellular alterations were scored as described by Hegde *et al.* (1982) [6].

Table No.1 Experimental protocol for CCl_4 induced hepatotoxicity

Group	Drug Treatment	Route and Dose (mg/kg)	Duration (Days)	Withdrawal of Blood and liver	Purpose
1	Honey	1 ml /animal p.o	$1^{st} – 14^{th}$	15^{th}	Control
2	CCl_4 + *Vediannabedhi Chendhooram*	1 ml / kg. s.c 23.4 mg / kg. p.o	13^{th} $1^{st} – 14^{th}$	15^{th}	Protective effect
3	CCl_4 + *Vediannabedhi Chendhooram*	1 ml / kg. s.c 50 mg / kg. p.o	13^{th} $1^{st} – 14^{th}$	15^{th}	Protective effect
4	CCl_4 + *Silymarin*	1 ml / kg. s.c 100 mg / kg p.o	13^{th} $1^{st} – 14^{th}$	15^{th}	Standard
5	CCl_4	1 ml / kg. s.c	13^{th}	15^{th}	Induce Liver damage

2.3.4.2. Statistical Analysis:

The data collected were subjected statistical analysis using unpaired t-test (P.S.S.Sundar Rao, J.Richard). The statistical significance of difference was taken as $P < 0.05$.

RESULTS AND DISCUSSION

3.1 Acute toxicity study:

No mortality, morbidity, weight loss or abnormal behavior was recorded after single exposure of the test compound @ 2000 mg/kg body weight during 14 days experimental period in Swiss Albino mice. This indicates that *Vediannabedhi Chendhooram* is safe up to a dose of 2000 mg/kg body weight.

3.2. Biochemical profiles of Transaminases:

In the present study SGOT, SGPT, ALP, Bilirubin, Total protein, Albumin and Globulin values were estimated by using a semi autoanalyser. Mean levels of SGOT, SGPT, ALP, Bilirubin, Total protein, Albumin and Globulin are presented in Table 2 and Table 3. Both the transaminases are referred to as "Makers of Cell Injury" (Loeb, 1982) and are excellent indicators of early hepatic lesion since they are the first to leak out from the cell in case of an injury [7]. Values of transaminases do not vary significantly with respect to age and sex in rat (Ringler and Debich, 1979).

In the present study the mean SGOT, SGPT, ALP, Bilirubin, Total protein, Albumin and Globulin value of each group of rats at the 15th day of the experiment is compared with the values of hepatotoxic control group. The results are demonstrated in **Tables 2&3**.

Table 2 Effect of *Vediannabedhi Chendhooram* in CCl_4 induced liver damage

Group	Percent change in body weight	Alkaline Phosphatase (ALP)	Aspartate Amino Transferase (SGOT)	Alanine Amino Transferase (SGPT)
I	13.33 ± 3.30	174.66 ± 20.35^{b}	190.66 ± 15.03^{NS}	61 ± 2.42^{a}
II	05 .00 ± 3.42	182.33 ± 14.37^{c}	171.33 ± 10.6^{NS}	62.33 ± 4.33^{a}
III	06.67 ± 3.33	220.83 ± 28.10^{NS}	219.5 ± 29.19^{NS}	77.5 ± 8.52
IV	13.33 ± 4.21	186.00 ± 23.12^{a}	226 ± 9.55^{NS}	66.5 ± 6.82^{b}
V	-06.67 ± 3.3	231.16 ± 07.26	217.5 ± 25.73^{NS}	88.67 ± 6.58

Values are mean ±S.E, Unpaired t – test. N = 6 in each groups.
^{a} P < 0.01 Vs Toxic ^{b} P < 0.05 Vs Toxic ^{c} P < 0.02 Vs Toxic

Table 3 Effect of *Vediannabedhi Chendhooram* in serum proteins and bilirubin levels

Group	Total protein	Albumin	Globulin	A / G ratio	Bilirubin
I	8.1 ± 0.2***	3.1 ± 0.13	4.36 ± 1.18	0.63 ±0.07	0.43 ±0.02
II	7.38 ± 0.2*	3.45 ± 0.12	3.93 ± 0.73	0.90 ± 0.18	0.36 ± 0.04
III	7.43 ± 0.2*	3.13 ± 0.17	4.3 ± 0.25	0.75 ± 0.08	0.42 ± 0.10
IV	8.45 ± 0.18***	2.9 ± 0.08	5.53 ± 0.24	0.53 ± 0.04	0.29 ± 0.02
V	6.63 ± 0.23	3.08 ± 0.08	3.85 ± 0.23	0.73 ± 0.02	0.38 ± 0.05

Values are mean ±S.E, Unpaired t – test. N = 6 in each groups.
*** P < 0.001 Vs Toxic * P < 0.05 Vs Toxic

In this study the rats included in Group V showed significant increase (P < 0.05) in ALP values compared to the values of Group I. In the group II (*Vediannabedhi Chendhooram* @ 23.4 mg/kg) there was significant reduction in ALP levels as compared to that of Group V (P < 0.02). In the group III (*Vediannabedhi Chendhooram* @ 50 mg/kg) there was non significant reduction in ALP levels as compared to that of Group V. In the group IV (*Silymarin* @ 100mg/kg) there was significant reduction in ALP levels as compared to that of Group V (P < 0.01). The values are presented in **Figure 1.**

The rats in Group V showed significant increase (P < 0.01) in SGPT values compared to the values of Group I. In the Group III there was non significant reduction in SGPT levels as compared to that of Group V. Whereas, in the Group II and IV there was significant reduction in SGPT levels as compared to that of Group V (P < 0.01). The values are presented in **Figure 2.** The SGOT levels showed non significant change in all the group of rats. The values are presented in **Figure 3**.

FIGURE 1 Effect of *VABC* on ALP levels

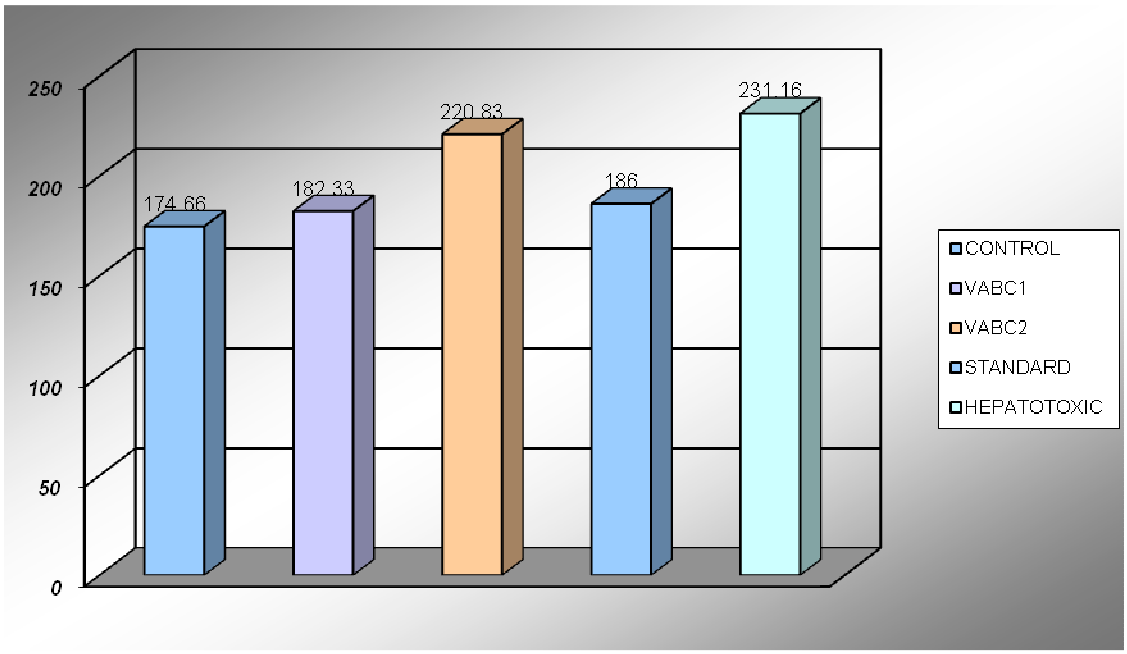


FIGURE 2 Effect of *VABC* on SGPT levels

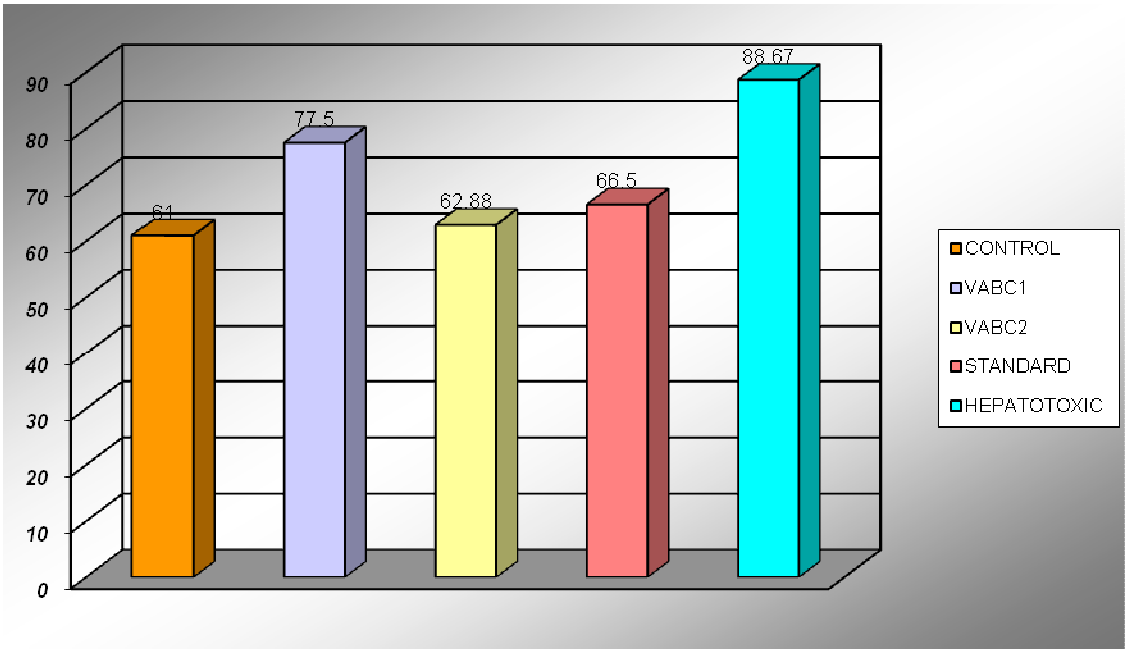
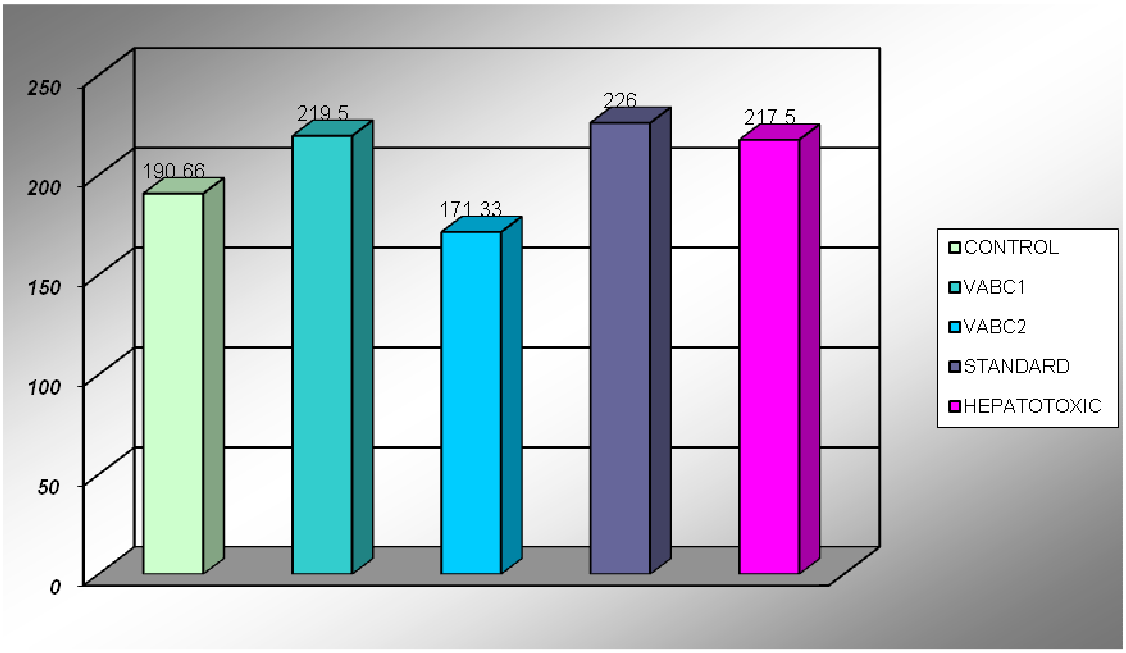
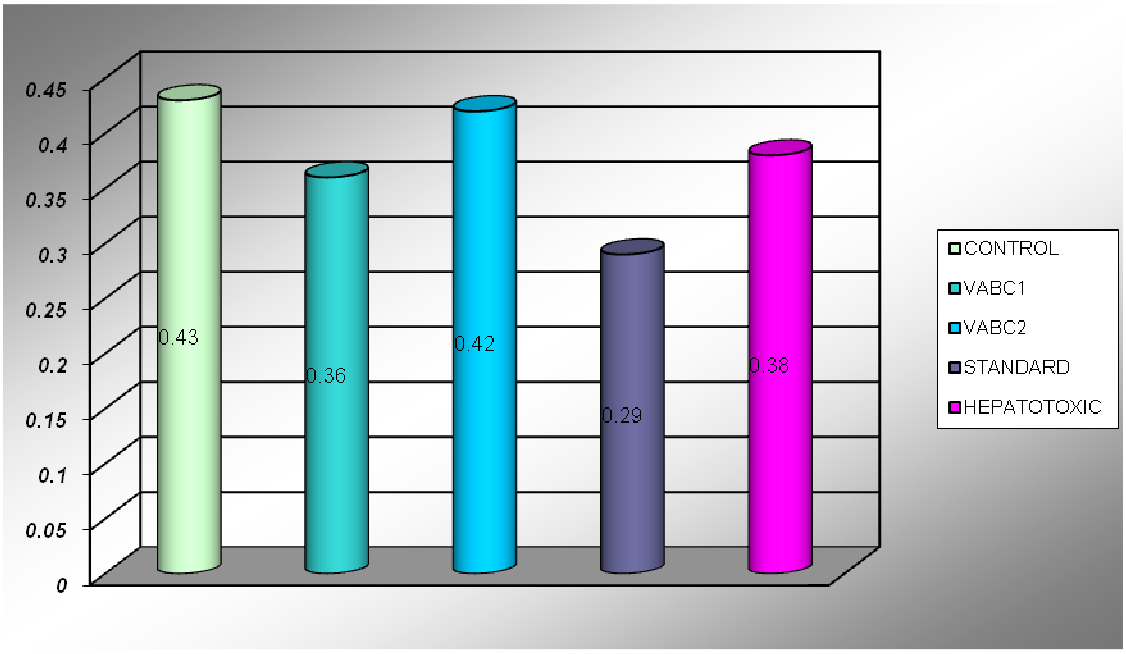


FIGURE 3 Effect of *VABC* on SGOT levels



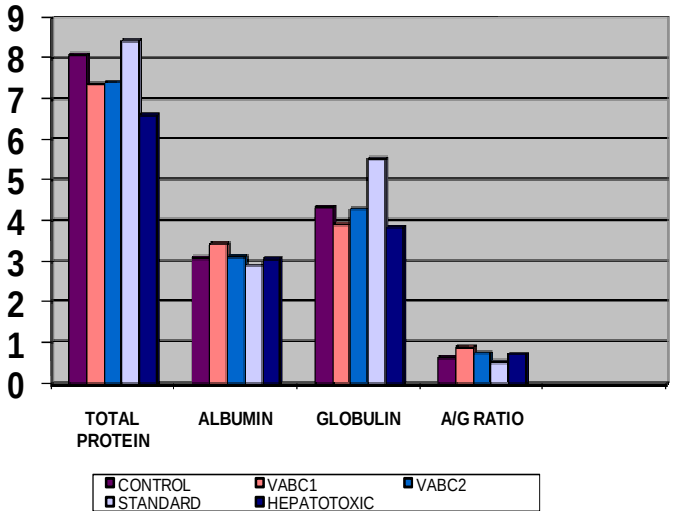
The effects of the drug on Serum Bilirubin levels in Groups II and Group III in comparison with the Standard, control and toxic Groups are demonstrated in **Figure 4,** with the values for evaluation.

FIGURE 4 Effect of *VABC* on serum bilirubin level



The rats in Group V showed significant decrease ($P < 0.001$) in Total protein levels compared to the values of Group I. In the Group II and III there was significant increase in Total protein levels as compared to that of Group V ($P < 0.05$). While, in the Group IV there was significant increase in Total protein levels as compared to that of Group V ($P < 0.001$). There was increase in serum globulin levels in treatment groups II, III and IV but they are statistically non significant. The values are presented in **Figure 5**. There was reduction in serum globulin levels in toxic group (Group V) but it was statistically non significant.

FIGURE 5 Effect of *VABC* on serum protein levels



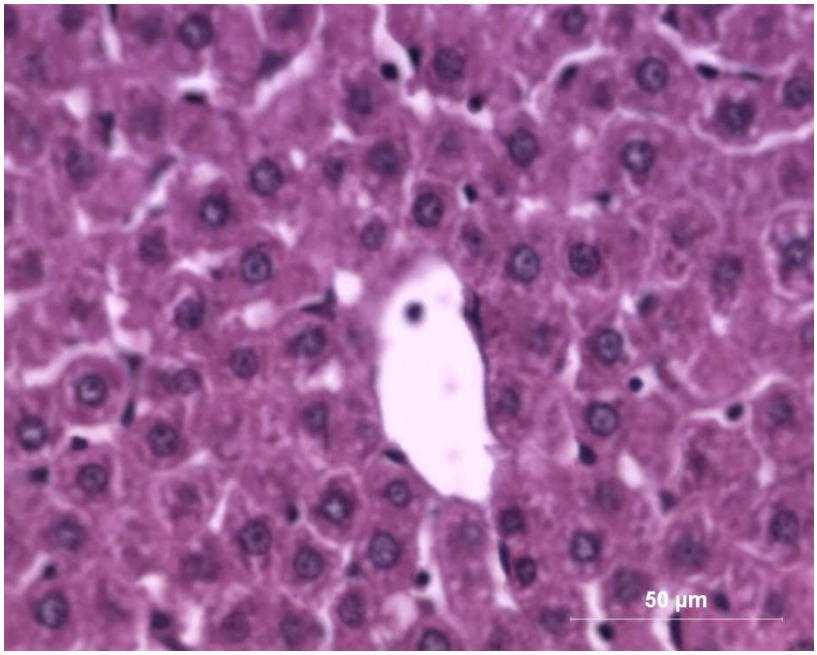
It obviously indicates that *Vediannabedhi Chendhooram* plays an important role in preventing and the liver toxicity produced due to carbon tetrachloride administration in rats.

3.3 Histopathological study of Hepatic sections

The present study was primarily aimed at carbon tetrachloride induced liver damage in the rats. Vacuolar, moderate and diffuse degeneration of hepatocytes, changes were observed in the livers of rats and no mortality was seen in experimental animals, during the experiment.

In the sections of liver obtained on 15th day of the experiment, there were no changes observed in normal control (Group I) whereas, mild degree of vacuolar degeneration of hepatocytes was observed in Group II (Fig.7) and IV (Fig.8). The severity of lesion increased (Moderate to Diffuse vacuolar degeneration) in Group III (Fig. 9) followed by Group V. The Photomicrographs are represented in **Figures 6 to 10**.

Fig. 6 Photomicrograph of rat liver in Group I (Normal Control showing normal section)



(H & E 320x)

Fig. 7 Photomicrograph of rat liver in Group II (*Vediannabedhi Chendhooram* @ 23.4 mg/kg). Section showing Mild vacuolar degeneration

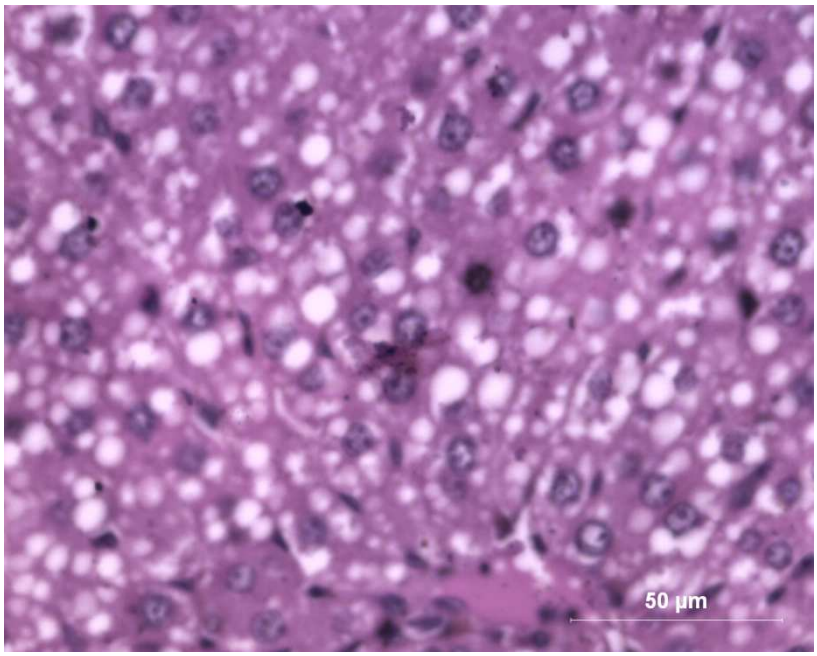


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Fig. 8 Photomicrograph of rat liver in Group III (*Vediannabedhi Chendhooram* @ 50 mg/kg). Section showing Moderate vacuolar degeneration

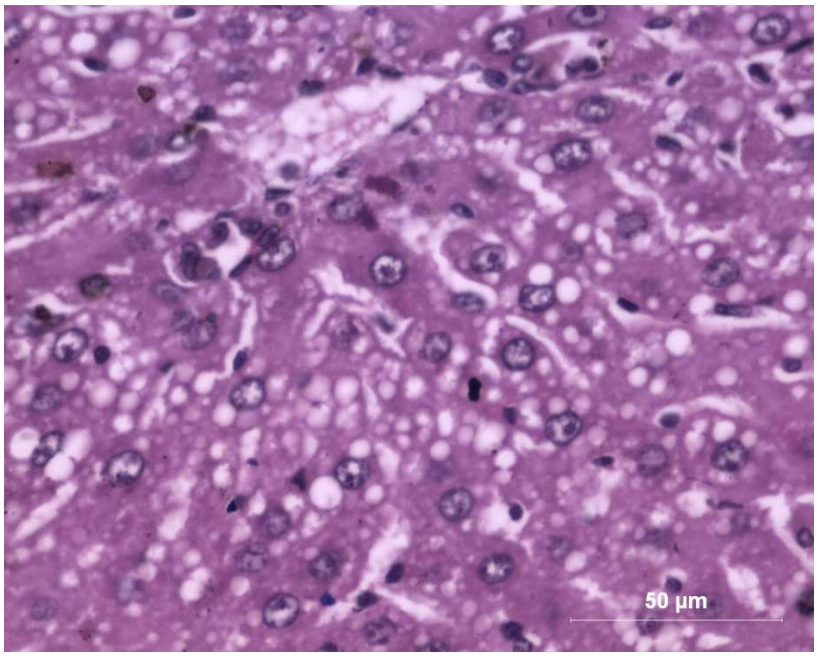

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Fig. 9 Photomicrograph of rat liver in Group IV (*Silymarin* @ 100 mg/kg)

Section showing Mild vacuolar degeneration

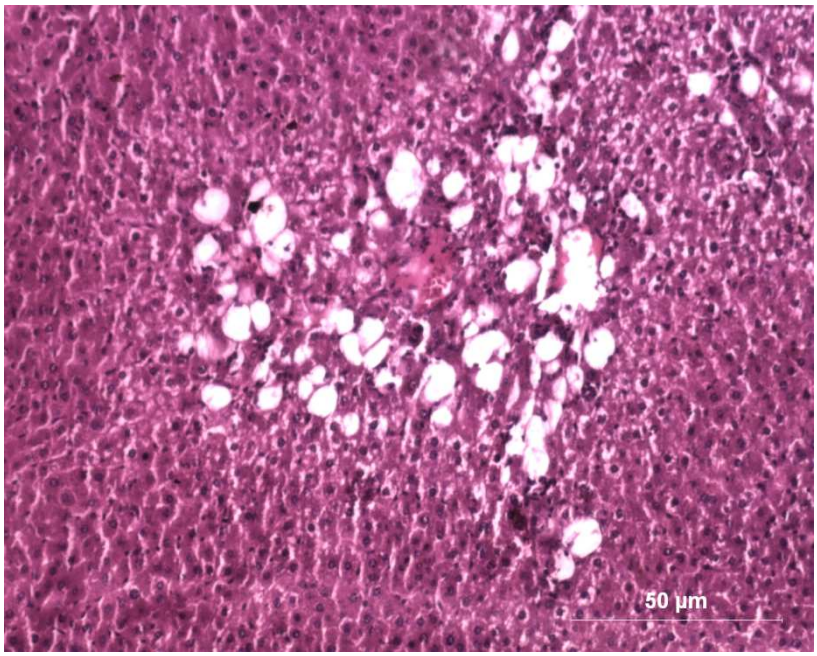

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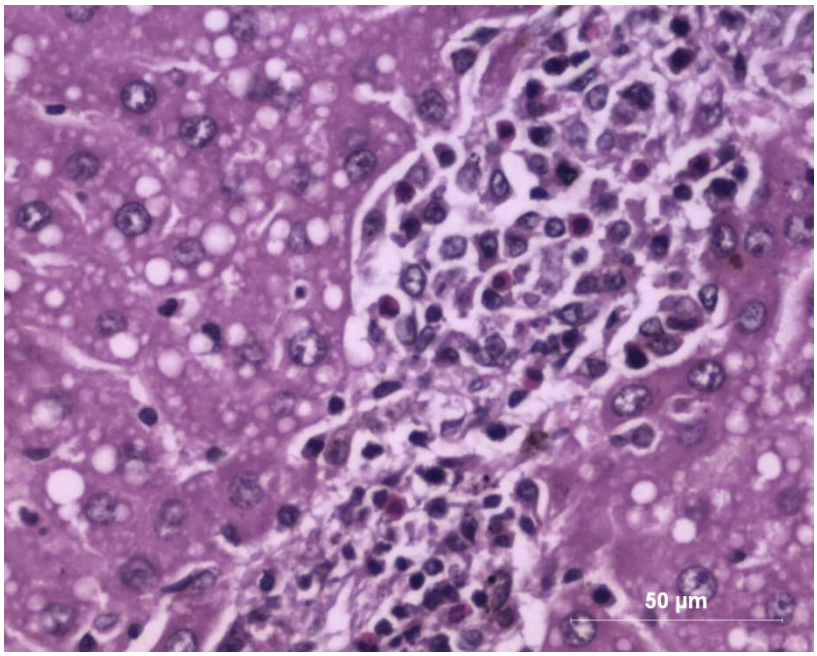
Fig. 10 Photomicrograph of rat liver in Group V (Hepatotoxic control)
Section showing Diffuse vacuolar degeneration



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The Biochemical analysis of the drug *Vediannabedhi Chendhooram* reveals the presence of minerals namely Selenium, Manganese, Sulfur, Iron, Calcium and Potassium. These are the minerals with antioxidant, free radical scavenging, haematinic activity, etc plays vital role in the management of Liver function.

Basically *Annabedhi* (Ferrous sulphate) used in this formulation has a specific role in Liver diseases which are frequently associated with haematological abnormalities; Anaemia of diverse etiology occurs in about 75% of patients with chronic liver disease. It also increases the power of digestion and Biliary secretion. *Vediuppu* one of

the salts used in the preparation of present drug having diuretic action also accounts for symptomatic relief in chronic case of hepatic damage with fluid retention in the body. Lemon juice containing antioxidants [8] which is used for grinding the above drugs also plays an important role in the management of symptoms like vomiting, giddiness, biliousness, etc. The literature evidences about the Siddha drug *Vediannabedhi Chendhooram* also strongly supports the hepatoprotective action of the drug.

Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of Hepatoprotective effect of drugs. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures [9]. CCl_4 produces an experimental damage that histologically resembles viral hepatitis in which liver necrosis is evident [10].

Elevated serum transaminases (AST and ALT) indicate the liver necrosis. Clinically, these enzyme levels are elevated in acute hepatitis, chronic hepatitis, chronic alcoholic hepatitis, diffuse intrahepatic cholestasis, extra hepatic obstruction and focal intrahepatic disease [11]. Serum level of Transaminases returns to normal once the healing of hepatic parenchyma and regeneration of Hepatocytes occurs [12]. The finding suggests the ability of VABC to regenerate Hepatocytes after CCl_4 intoxication.

Alkaline phosphatase (ALP) is the prototype enzyme that reflects the pathological alteration in Biliary flow [13]. Clinically, this enzyme indicates diffuse intrahepatic cholestasis, extra hepatic obstruction and focal intrahepatic disease. CCl_4 induced elevation of this enzyme in the serum is in line with high level of serum bilirubin content. Elevated total bilirubin is a sign for acute hepatitis, chronic hepatitis, chronic alcoholic hepatitis, diffuse intrahepatic cholestasis and extra hepatic obstruction cholestasis [11]. The *VABC* mediated reduction in the increased serum ALP level with the concurrent depletion of raised bilirubin suggests the possibility of the test drug to stabilize Biliary dysfunction in rat liver during hepatic injury. The *VABC* reversed the elevated levels of Transaminases, ALP and total Bilirubin which indicate the healing of damaged hepatic cells.

Conclusively the study in rat models using Wistar albino rats demonstrates liver damage in toxic groups, evidenced by elevated serum enzyme levels and histopathological features, the protective groups administered with *Vediannabedhi Chendhooram* @ 23.4 mg/kg along with Carbon tetra chloride showed protective effects on liver damage evidenced by reduction in serum enzyme levels and histopathological studies. From the biochemical analysis and histopathological studies it was observed that *Vediannabedhi Chendhooram* is effective at the rate of 23.4 mg orally in rats as compared to other groups.

Further, the hepatoprotective and curative effect of the drug on chronic liver damage have to be studied. Its effect on lipid peroxidation in liver and antioxidant enzyme status in liver during treatment with *Vediannabedhi Chendhooram* have to be evaluated.

CONCLUSION

Pharmacological studies showed that the drug has significant hepatoprotective activity at the dose of 23.4 mg and no significant adverse effects. The drug is more effective in the therapeutic dose determined by *Siddhars* (*Oru kuntri edai i.e, 150 mg*) and less effective in higher doses. Hence it can be concluded that the correct dose regimen, *anupanam* (adjuvant) and exact method of preparation of drug only give expecting results and more success to Siddha Medicines.

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ABBREVIATIONS:

VABC	*VediAnnabedhi Chendhooram*
AAS	Atomic Absorption Spectrometer
XRF	X-ray Fluorescence
XRD	X-ray Diffraction
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
ALP	Alkaline Phosphatase
CCl_4	Carbon Tetra Chloride

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