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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF WHOLE PLANT VENTILAGO MADERASPATANA

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ABSTRACT
The present study was aimed at establishing evaluation of hepatoprotective activity of 90% ethanolic extract of whole plant of Ventilago maderaspatana in carbon tetra chloride induced hepatotoxicity in albino rats .The hepatoprotective activity was assessed by thiopental induced sleep model. Dose selection was based on acute toxicity studies (1000ml/kg body weight).SGPT, SGOT, ALP, Total bilirubin, serum albumins was measured. Statistical tests like Annova and Dunnet's t-test was used. Observed data was statistically significant($p<0.001$) in reducing blood SGPT,SGOT,ALP, Total bilirubin and increasing the total albumins and body weight .The hepatoprotective activity was compared with that of standard LIV-52(hepato protective drug)

Key words: *Whole plant of Ventilago Maderaspatana , Biochemical investigation,LIV-52, Thiopental induced sleep.*

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INTRODUCTION

Ventilago maderaspatana: Family: Rhamnaceae
Ventilago refers to the whole herb of Ventilago maderaspatana, a herb of the family Rhamnaceae (6). Leaves are pale green, shrubs are scandent. Branchlets are brownish, pubescent. Stipules caducous; petiole 4-7 mm; leaf blade abaxially pale green, adaxially dark green, shiny, ovate-oblongto ovate-elliptic(7).
Ventilago is distributed in Forests of low elevations- South Greece, India, Indonesia (Java), Myanmar, Sri- lanka.

It is believed that Ventilago maderaspatana possesses the effects of Kapha, Dyspesia, Colic Disorder, Leprosy, Scabies, Pruritis, and other Skin fever and General disability(8).
Keeping in view all the literature survey the present study was planned to verify the claims that the Ventilago maderaspatana is highly useful as Hepatoprotective. Hence an attempt is made to compare its Hepatoprotective potential with that of established marketed Hepatoprotective. An attempt is also made to evaluate its effect on liver metabolic functions.
The plant is reported to contain alkaloids, carbohydrates, non steroidal saponins. 90% ethanolic extract of whole plant *Ventilago*

maderaspatana evaluated for hepatoprotective activity.

OBJECTIVE :

The present study aims at evaluating hepatoprotective activity of *Ventilago maderaspatana* in carbon tetrachloride induced liver damage taking Thiopental induced sleeping time as the criteria .The Biochemical estimation of SGOT, SGPT, ALP, Total bilirubin ,total proteins and morphological Studies.

MATERIAL :

Ventilago maderaspatana (whole plant extract) was sourced and obtained from Department of Botany, S.V. University. The identification was made on botanical and pharmacological basis. The macroscopical study of drug was conducted with naked eye. The size, shape, colour and organoleptic characters were observed and plant was confirmed on the basis of literature description.

Chemicals

- CCl_4 for inducing Hepato-toxicity
- Liv-52 (standard drug).
- Thiopentol
- Ventilago maderaspatana
- Liquid Paraffin
- Normal saline

Animals:

Albino rats (wistar strain) weighing 150-200g and Albino mice weighing 20-25gm of either sex were used in this study. They were housed in polypropylene cages and maintained at 27˚c± 2˚c. under 12 hours dark/ light cycle. They were fed with standard rat feed (Hindustan lever Ltd.) water and libitum was provided under hygienic conditions.

EXTRACTION OF DRUG:

Preparation of plant extract: Plant of Ventilago maderaspatana are shade dried at room temperature and is powdered mechanically.The dried powder is defatted with petroleum Ether and 150 gm of powdered material was refluxed with 500ml of 90% ethanol and 50ml of distilled water for 18-24 hours using Soxhlet Apparatus. The semisolid mass was then spread on the porcelain tray and dried. The dried mass was scrapped and powdered. The powder was sieved and stored in an airtight container

PhytoChemical Screening

Chemical test	**Ethanol extract**

Test for Alkaloids	**RESULT**
a) Mayers' test	POSITIVE
b) Dragendroff's test	POSITIVE
c) Wagener's test	POSITIVE
d) Hager's test	POSITIVE

Test for Carbohydrates	**RESULT**
a)Fehlings' test	POSITIVE
b)Molisch' test	POSITIVE
c)benedict's	POSITIVE
d)barforde's	POSITIVE

Test For Saponins	**RESULT**
A) Foam Test	POSITIVE
B)Heamolysis	POSITIVE

Test for steroidal saponins	RESULT
a) Salkowski's test	NEGATIVE
b) libermamm's – buchard's test	NEGATIVE

Animal grouping : Control group – normal saline (1ml/kg), **Hepatotoxic control**- CCl_4(1.5ml/kg) **Plant used** - *Ventilago maderaspatana* (1000ml/kg) , **Standard drug**- LIV52 (0.216ml/kg) Later thiopental 50mg/kg was injected and duration of sleep was noted

RESULTS AND DISCUSSION

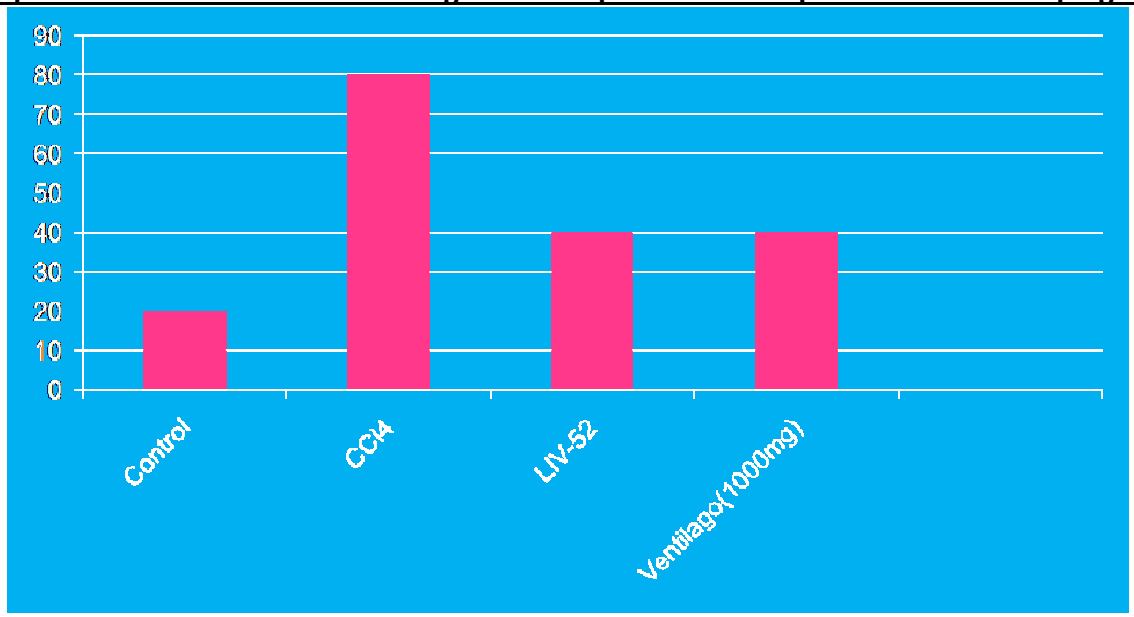
The sleeping time of the four groups of animals are summarized in the following table.

Treatment	Sleeping Time Mean ± SEM (min)
Normal Control (1ml normal saline)	15.513 ± 0.512
CCl_4 (1.5ml/kg i.p.)	84.99 ± 1.510
Ventilago maderaspatana 1000mg/kg + CCl_4 (1.5ml/kg i.p.)	46.021 ± 1.800 ***
LIV-52 0.216ml/kg + CCl_4 (1.5 ml/kg i.p.)	40.66 ± 1.12 ***

Values are the Mean ± SEM of six mice/treatment ***Significance P<0.001 compared to CCL4 treatment.

ANOVA, Dunnet's't' tes

Graphical Representation Of Effect of Ventilago maderaspatana on Thiopental induced sleeping time:



RESULTS

Acute toxicity:

No mortality was found in any of the 3 groups of animal so 1000ml/kg Ventilago maderaspatana was considered as standard drug preparation.

Effect of Ventilago maderaspatana on Thiopental induced sleeping time:

Effects of Ventilago maderaspatana on liver enzymes in CCl4 induced hepatotoxicity:

SGOT level increased significantly in Hepatotoxic (CCl4) group i.e. (373.67± 20.250). Ventilago maderaspatana 1000mg/kg reduced the elevated levels of SGOT to (271.00 ±14.245), which is near to Liv-52 effect (258.00 ± 12.367).

There was an increase in **SGPT** levels in Hepatotoxic (CCl4) treated group (181.67 ±7.856), SGPT levels were restored to (81.000±4.344) by 1000mg/kg Ventilago maderaspatana, Whereas (74.333±4.224) was the effect of 0.216ml/kg Liv-52.

ALP levels observed in CCl_4 treated group (410.17 ± 12.240) were higher than that of Ventilago maderaspatana 1000mg/kg i.e. 245.33 ± 22.028. The value in the LIV-52 0.216ml/kg treated group was 229.50 ± 18.273.

In case of **Total Bilirubin,** there was increase in the CCl4 treated group (1.550 ± 0.1118), while Ventilago maderaspatana 1000mg/kg reduces the levels of total bilirubin 0.9917 ± 0.1068 which is closed to that reduced by LIV-52 0.216ml/kg (0.7067 ± 0.06667)

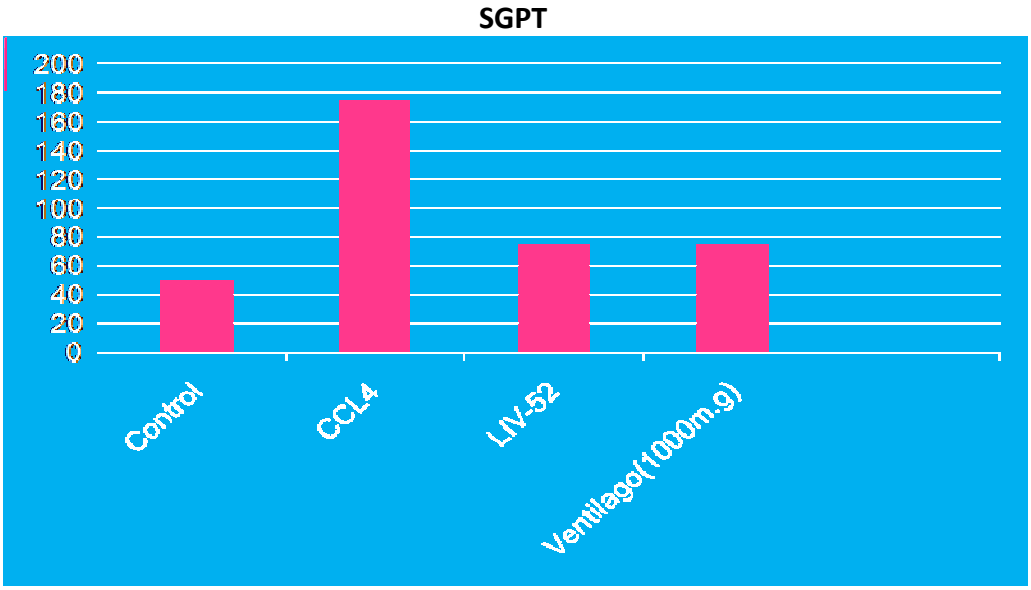
The effect of Ventilago maderaspatana on liver enzymes in CCl4 induced hepatotoxic group are summarized in the following table.

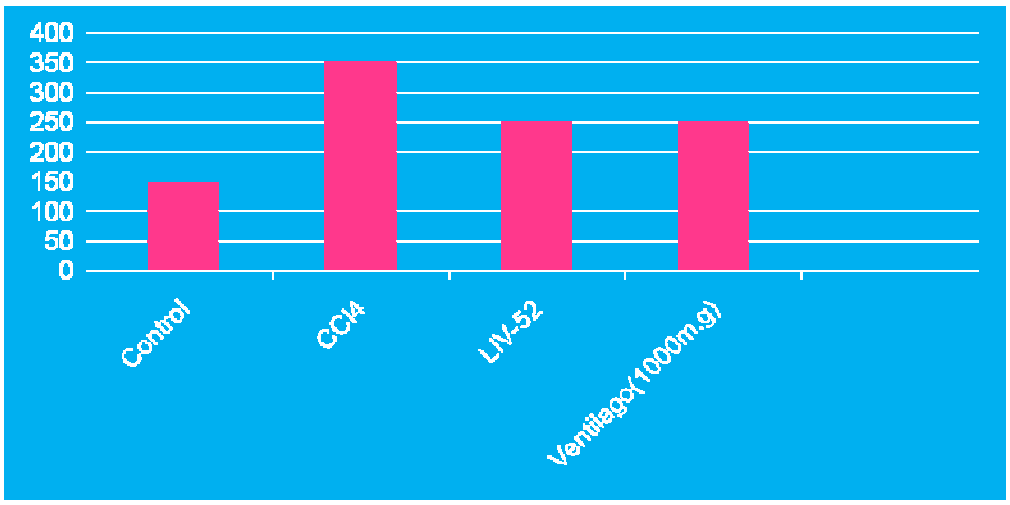
	BIOCHEMICAL PARAMETERS MEAN ± SEM					
TREATMENT	**SGPT**	**SGOT**	**ALP**	**Total Bilirubin**	**Serum Albumin**	**Serum Proteins**
	U/L	**U/L**	**IU/L**	**mg/dl**	**g/dl**	**g/dl**
Normal Control (1ml distilled water i.p. + 1ml/kg liquid paraffin i.p.)	47.666 ± 5.506	131.666 ± 7.719	66.333 ± 5.572	0.7483 ± 0.0744	4.1 ± 0.0894	6.8 ± 0.1571
CCl_4 Control (1.5ml/kg i.p)	181.67 ± 7.856	373.67± 20.250	410.17 ± 12.240	1.550 ± 0.1118	2.217 ± 0.07032	4.033 ± 0.1820
Ventilago maderaspatana (1000mg/kg) + CCl_4 (1.5ml/kg i.p.)	81.000± 4.344	271.00 ± 14.245**	245.33 ± 22.028***	0.9917 ± 0.1068**	3.333 ± 0.1202**	5.883 ± 0.4135**
LIV-52(0.216ml/kg) + CCl4 (1.5ml/kg i.p.)	74.333± 4.224	258.00 ± 12.367**	229.50 ± 18.273***	0.7067 ± 0.06667**	3.483 ± 0.1327**	5.483 ± 0.2007**

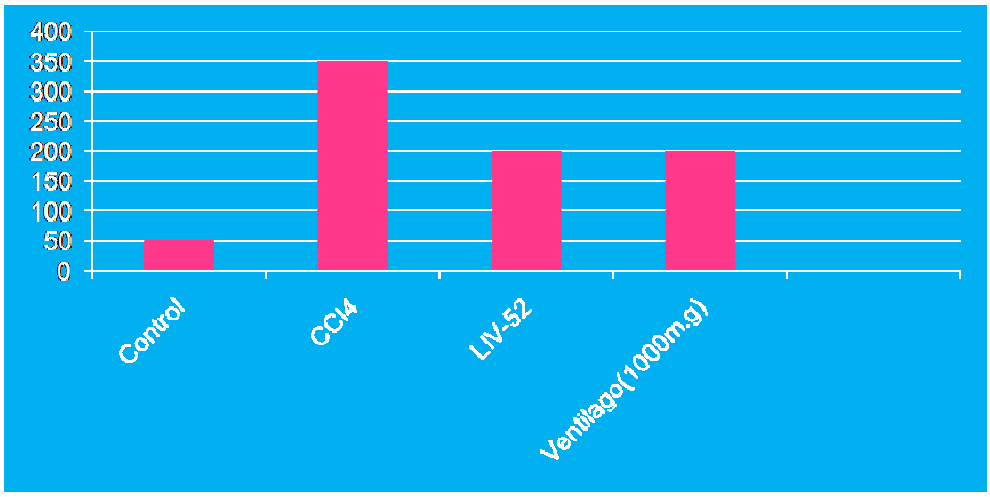
Values are the Mean ± SEM of six rats/treatment

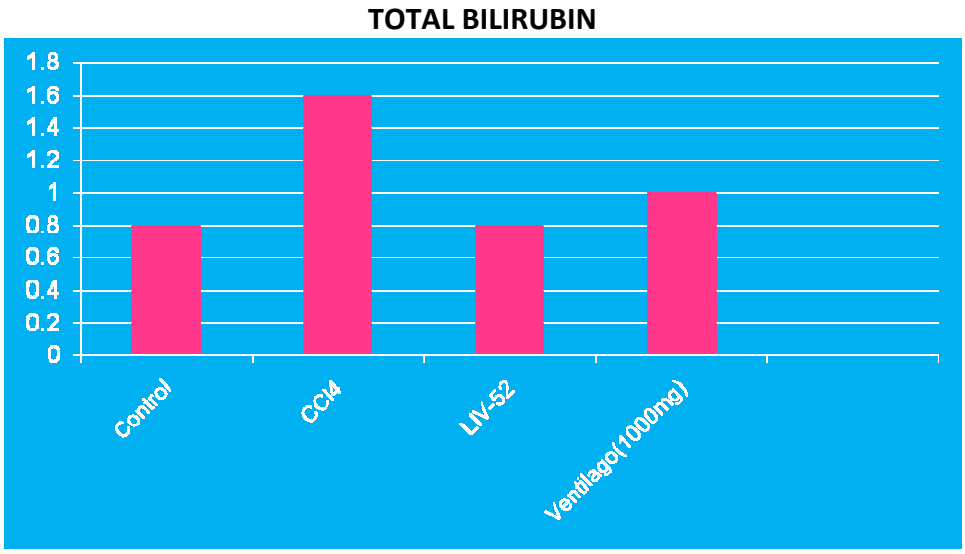
***Significance P<0.001 compared to CCl_4 treatment. ANOVA, Dunnet's't' tes

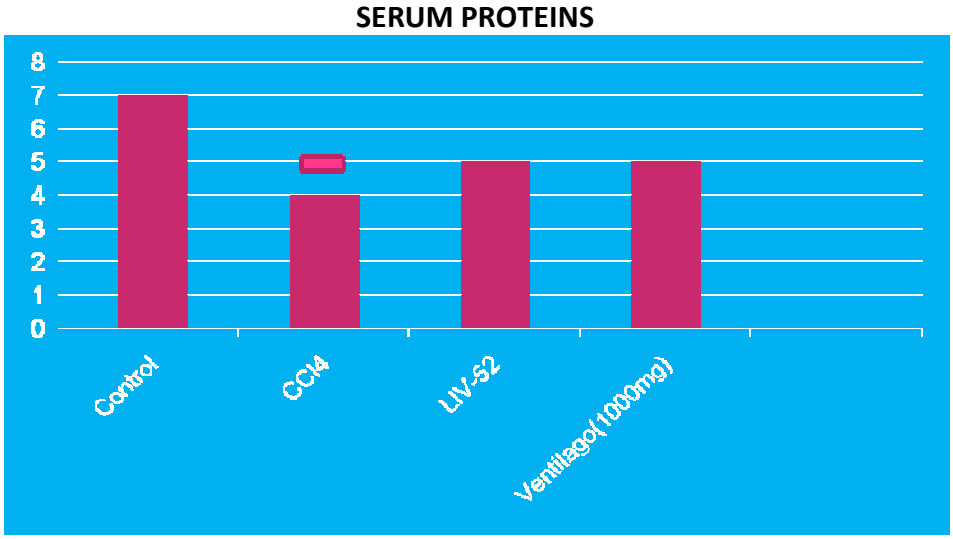
Graphical Representation Of Effect of Ventilago maderaspatana on liver enzymes in CCl4 induced Hepatotoxicity:











Morphological studies:

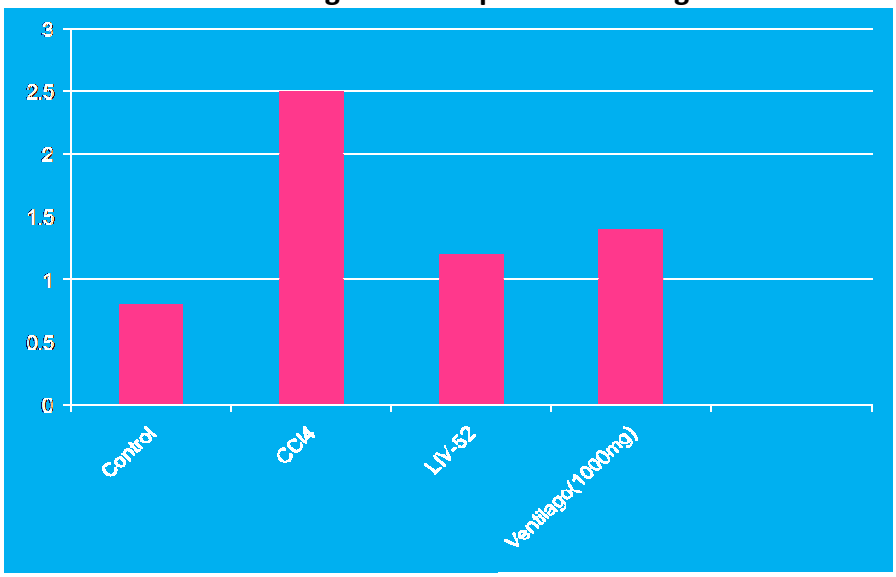
Weight Variation Test:

The animal were sacrificed after collection of blood sample, Liver was removed and was washed with cold saline solution The liver is pressed between filter paper and then weight.

Treatment	Weight In Grams Mean ± SEM (gm)
Normal Control (1ml normal saline)	0.86 ± 0.915
CCl_4 (1.5ml/kg i.p.)	2.8 ± 1.08
Ventilago 1000mg/kg + CCl_4 (1.5ml/kg p.o)	1.46± 1.210 ***
LIV-52 0.216ml/kg + CCl_4 (1.5ml/kg i.p.)	1.48 ± 1.823 ***

Values are the Mean ± SEM of six rats/treatment ***Significance P<0.001 compared to CCL4 treatment

Graphical Representation Of Effect of Ventilago maderaspatana on Weight of Liver damaged by CCl4:



Histopathological Studies in CCl4 induced hepatotoxicity:

Group-I: In case of control group, central vein, hepatic globular structure, portal tract and kupffer cells were normal.

***Suggestive*:** Normal Liver.

Group-II: In the case of carbon tetrachloride treated group, Liver sinusoids were congested. Hepatic globular architecture was normal, hepatic cells showed various degree of fatty degeneration like ballooning of hepatocytes, fatty cysts, infiltration of lymphocytes and proliferation of kupffer cells.

***Suggestive*:** Fatty liver.

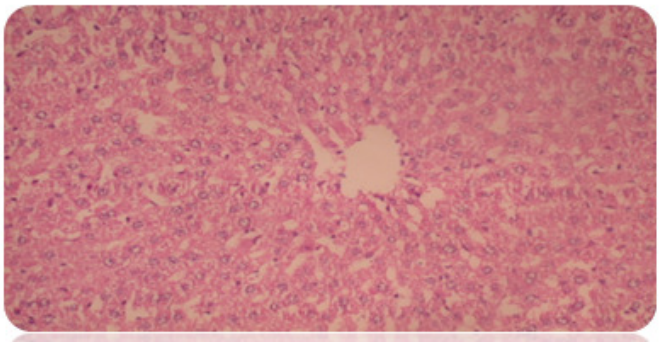
Group-III: In the case of 1000mg/kg Ventilago treated group the hepatic globular architecture was normal. There were occasional fatty cells, areas of lymphocytic infiltration and kupffer cells proliferation. (Fig no. 29)

***Suggestive*:** Normal liver with occasional lymphocytic infiltration.

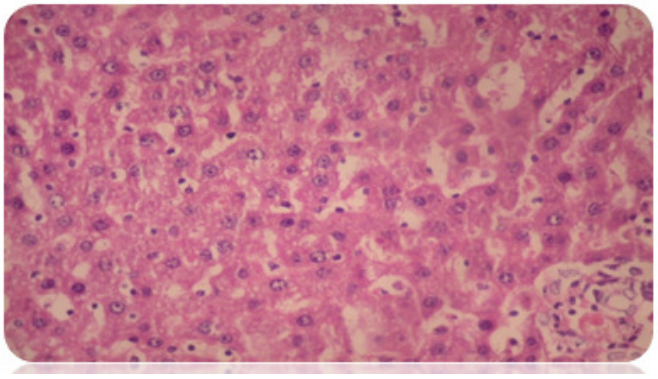
Group-IV: In the case of 0.216ml/kg LIV-52 treated group majority of hepatocytes are normal the hepatic globular architecture was normal. A few areas shown lymphocytic infiltration.

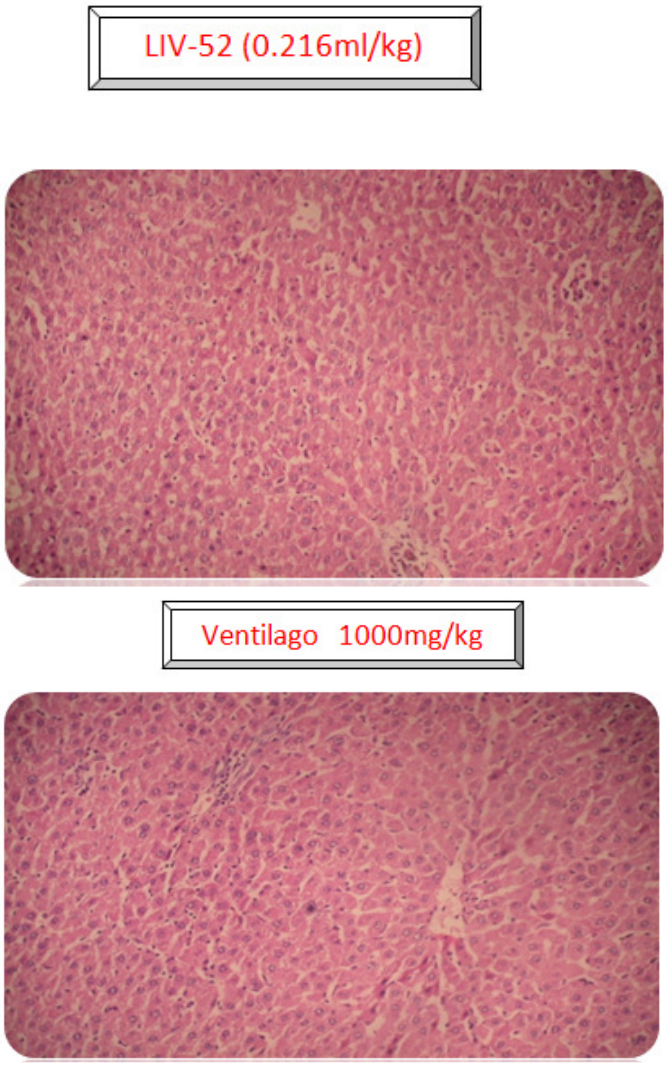
***Suggestive*:** Normal liver with occasional lymphocytic infiltration.

NORMAL



CCl4





DISCUSSION

The normal physiological functioning of liver is to metabolize various endogenous and exogenously administered chemicals so as to terminate or inactivate these agents. Liver protects the whole body from the various environmental, chemical challenges. In addition liver has inbuilt mechanism to protect itself and to regenerate. On several occasions these hepatotoxic challenges overpowers inbuilt protective mechanism and cause Hepatotoxicity resulting in the hepatic necrosis and hepatitis.

Ventilago maderaspatana is one such herb being used since ancient times for the treatment of various ailments. Upon literature review it was found that the phytochemical and pharmacological profile of this plant is complete.

Ventilago maderaspatana was subjected to screen for hepatoprotective activity against CCl_4 induced Hepatotoxicity in rats. Administration of CCl_4 has caused the Hepatotoxicity as evidenced by the enhanced levels of biochemical markers of Hepatotoxicity, e.g. SGPT, SGOT, ALP, Bilirubin and reduction in total proteins.

Histopathological reports reveal that administration of CCl_4 had caused degeneration of fatty cysts, infiltration of lymphocytes, proliferation of kupffer cells and congestion of liver sinusoids. This further confirms that CCl_4 administration causes Hepatotoxicity. This is in conformation with the earlier reports. Upon Pre-treatment with Ventilago maderaspatana there was decrease in the elevated levels of biochemical marker like SGPT, SGOT, ALP, Total bilirubin and decrease in to total protein levels. Similarly, histopathological observations show that hepatic globular architecture was normalized, fewer lymphatic infiltration was seen and kupffer cells proliferation appeared to be normal. This observation suggests that Ventilago-maderaspatana possess hepatoprotective activity against CCl_4 induced Hepatotoxicity.

Thiopental sodium is an ultra short acting barbiturate which induces sleep in mice. Thiopental is metabolized in liver by the hepatic cytochrome P 450. Upon treatment with CCl_4 prolongation of sleeping time was noted.

The prolongation of sleeping time was found to be partially reversed by pretreatment with Ventilago maderaspatana and markedly reversed by pretreatment with LIV-52. This above result shows hepatoprotective profile of Ventilago maderaspatana. Apart from this, efforts are required to evaluate the effect of Ventilago maderaspatana on liver metabolic functions.

CONCLUSION

Carbon tetrachloride induced hepatotoxicity model was used to assess the hepatoprotective activity of Ventilago maderaspatana. Hepatotoxicity was confirmed by rise in biochemical markers of liver (SGOT, SGPT, ALP) and hepatic MDA levels. Ventilago maderaspatana pretreatment effectively

prevented hepatic damage, by reducing the elevated enzymes levels. The hepatoprotective effect of this agent may be due to inhibition of lipid peroxidation as indicated by reduced levels of LDL in Ventilago maderaspatana treated group. Similar findings were also observed with LIV-52.

Histopathological studies also showed that carbon tetrachloride caused hepatic injury. Pretreatment with Ventilago maderaspatana exhibited protection which was comparable to that of LIV-52.

Sleeping time was prolonged by thiopental-sodium, in mice treated with carbon tetrachloride is an indication of deteriorated hepatic function. As it was noted that Ventilago maderaspatana and LIV-52 treated groups showed shorter sleeping time as compared to carbon tetrachloride treated group indicating the reversal of hepatic dysfunction.

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