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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF THE EXTRACT VICOA INDICA (L) AGAINST CCL$_4$-INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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Abstract:

The current study intended to determine the hepatoprotective activity of an extract of Vicoa indica (VI) leaves using recognized rat models. Seven groups of rats (n = 5) were divided and Group I treated an administration of Saline 1ml/Kg and Olive oil (3ml/Kg) weekly twice orally for 4 weeks (control), Group II induction of hepatotoxicity using carbon tetrachloride CCl_4 30% in olive oil (3 ml/Kg) oral route, Group III Silymarin 100mg/Kg at oral route weekly twice for 4 weeks. Group IV CCl_4 30% in olive oil (3 ml/Kg) oral route, Ethyl acetate extract low dose at oral route weekly twice for 4 weeks, Group IV CCl_4 30% in olive oil (3 ml/Kg) oral route, Group V Ethyl acetate extract high dose at oral route weekly twice for 4 weeks, Group VI CCl_4 30% in olive oil (3 ml/Kg) oral route, Methanol extract low dose at oral route weekly twice for 4 weeks CCl_4 30% in olive oil (3 ml/Kg) oral route, GroupVII Methanol extract high dose at oral route weekly twice for 4 weeks. After last dosing Blood samples and liver specimens were collected for biochemical and histopathological analysis. The result was revealed that VI exhibited a significant ($p < 0.05$) hepatoprotective activity against both inducers and test group compared with standard group which could be related to their phytochemical constituents and antioxidant property of the extract.

Key words: *Silymarin, hepatoprotective, phytochemical, Vicoa indica. antioxidant*

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INTRODUCTION:

The liver is a largest organ in the human body that is responsible for a collection of functions that help support metabolism, immunity, digestion, detoxification, vitamin storage among other functions. It covers around 2% of an adult's body weight. Liver has an essential role in regulation of physiological processes in living system. Liver diseases are among the most serious illness and life threatening disease such as acute and chronic hepatitis (inflammatory liver diseases), secondly hepatosis (non- inflammatory diseases) and finally cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mostly formed by the exposure of toxic chemical substances like certain antibiotics, chemotherapeutic agents, peroxidised oil, carbon-tetrachloride, chlorinated hydrocarbons and also excessively drinking of alcohol, viral infections and autoimmune/disorder [1].

Liver diseases are a common health problem in universal, and the conventional medicines are used in the treatment of hepatic diseases are occasionally insufficient and can have some severe adverse effects .Therefore, the mode of treatment would be shifted to medicinal plants as new bases of hepatoprotective agents [2] . Medicinal plants and its metabolites play a key role in the human health care system. About 70-80% of the world population trust on the use of traditional herbal medicine which is mainly based on plant products.

Vicoa indica is a medicinal herbal plant belonging to the family Compositae. Leaves are alternate arrangement and 7-12 x 2-3.5 cm length. *V.indica* is elliptic ovate in shape, acuminate at apex area, attenuate at base part, margins are serrate, hairy above and on nerves below, white cottony between the prominently reticulated veins below and upper leaves sessile to marginally petioled, lower ones of *V.indica* is long petioled. Corolla campanulate is in purple colour and tube slender is 0.5-0.6 cm long, widened at mouth region. Achenes of *V.indica* are 0.1-0.2 cm long and barbellate of *V.indica* hairs are 0.25-0.3 cm (*long.www. kerala plants.in*) *V.indica* is used by tribal population in northern states of India. It acts as a contraceptive agent and used as female anti-fertility drug. The ethnobotanical assessments showed the infusion of whole plants was used in abortion [3], *V.indica* roots are therapy for cough and jaundice [4]. In this present study *V.indica (*herbal plant) leaf extract is used to evaluate the hepatoprotective activity in rat model.

MATERIALS AND METHODS:

Collection of Plant Materials:

The *Vicoa indica* leaves were collected from the Kanyakumari district, Tamil and, India. The plant was authenticated by Mr. Chelladurai, Research Botanist (Rtd), CCRAS Tirunelveli, Tamil Nadu.

Preparation of extracts:

About 1 kg of air-dried leaves of plant was extracted in sox let assembly methanol 70% and ethyl acetate. The extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The color and consistency of the extract were also noted. All the solvents used for this entire work were of analytical reagent grade (Merck, Mumbai). The yield of the extract was 27.46 % and 23.31% (w/w). In each experiment, the extract was diluted with water to desired concentration.

Animals:

Adult male albino rats weighing about 200-250g were used in this study. Rats were maintained in clean, sterile, polycarbonate cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. This study was approved by IAEC of Cape Bio Lab & Research Centre, CSI Complex, Marthandam – 629 165 and the study approval number is **CBLRC/IAEC/06/01 – 2020.**

Statistical analysis:

The results are presented as mean ± standard error of mean and analyzed using the one-way analysis of variance test with the Dunnet post-hoc test, with $p < 0.05$ as the limit of significance.

Qualitative chemical tests:

The methanol and ethyl acetate extract subjected to qualitative chemical analysis to test the presence of alkaloids, carbohydrates, proteins and amino acids, phytosterols, glycosides, saponins, flavonoids, triterpenoids and fixed oils [5] [6].

Hepatoprotective activity:

Grouping of animals

Seven groups of animals containing six animals in each group were divided for the hepatoprotective activity in rat models.

Groups	Treatment 28 Days
Group I	Saline 1ml/Kg and Olive oil (3ml/Kg) weekly twice orally for 4 weeks
Group II	CCl_4 30% in olive oil (3 ml/Kg) weekly twice orally for 4 weeks
Group III	CCl_4 30% in olive oil (3 ml/Kg) oral route Silymarin 100mg/Kg of *VI* at oral route weekly twice for 4 weeks
Group IV	CCl_4 30% in olive oil (3 ml/Kg) oral route, Ethyl acetate extract 100mg/Kg of *VI* at oral route weekly twice for 4 weeks
Group V	CCl_4 30% in olive oil (3 ml/Kg) oral route, Ethyl acetate extract 200mg/Kg of *VI* at oral route weekly twice for 4 weeks
Group VI	CCl_4 30% in olive oil (3 ml/Kg) oral route, Methanol extract 100mg/Kg of *VI* at oral route weekly twice for 4 weeks
Group VII	CCl_4 30% in olive oil (3 ml/Kg) oral route, Methanol extract 200mg/Kg of *VI* at oral route weekly twice for 4 weeks

After 24 hours of the last day of treatment, all the animals were weighted and collected the blood samples for the measurement of lipid profile and liver enzymes. After collection of blood samples the animals were sacrificed under euthanasia and Liver was removed from the each animal, weighted the liver specimen and perfused in ice-cold saline solution and evaluated the liver enzyme parameters and carried out the histopathological studies.

RESULTS:

V. indica leaves extracts were subjected to qualitative chemical tests for the detection of various phytoconstituents such as alkaloids, carbohydrates, proteins and amino acids, glycosides, flavonoids, tannins, phenolic compounds, saponins. The phytochemical screening results are shown in Table1.

Table 1: Qualitative Chemical Analysis of Phytoconstituents of the methanol and ethyl acetate Extract of *V. indica*

S. No.	Tested Components	*EAV.indica*	*MEV.indica*
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Glycosides	+	+
4.	Terpenoids	-	-
5.	Proteins	+	+
6.	Amino acids	+	+
7.	Steroids	+	+
8.	Flavonoids	+	+
9.	Phenols	+	+
10.	Tannins	+	+
11	Saponins	+	+

+ = Presence - = Absence

Hepatoprotective activity:

The result of methanol and ethyl acetate leaf extracts of *V. indica* is exhibited that a dose depended Hepatoprotective activity in rats and the methanol extract showed a better response than ethyl acetate extract as compared to standard and control group.

Table: 2 Effect of methanol and ethyl acetate extracts of *V. indica* on Hepatoprotective activity

Groups	Treatment	Observation in liver enzymes	
		AST (U/L)	**ALT (U/L)**
I	Saline 1ml/Kg and Olive oil (3ml/Kg)	109.23±10.32	62.43±5.34
II	CCl_4 30% in olive oil (3 ml/Kg)	1643.31±76.43	812.62±64.23
III	CCl_4 30% in olive oil (3 ml/Kg) + Silymarin 100mg/Kg	642.45±32.12*	473.54±16.12*
IV	CCl_4 30% in olive oil (3 ml/Kg) + Ethyl acetate extract of *VI* 100mg/Kg	1256.32±87.12	765.21±45.43
V	CCl_4 30% in olive oil (3 ml/Kg) + Ethyl acetate extract of *VI* 200mg/Kg	856.5±23.13*	645.42±36.13*
VI	CCl_4 30% in olive oil (3 ml/Kg) + Methanol extract of *VI*100mg/Kg	1124.12±112.32*	678.56±42.34*
VII	CCl_4 30% in olive oil (3 ml/Kg) + Methanol extract of *VI* 200mg/Kg	764.43±43.23*	584.34±35.12*

VI- V. indica

Values are expressed as mean ± standard mean error. *Data differed significantly at $p < 0.05$ when compared with the normal control group and standard group.

Histopathological evaluation:

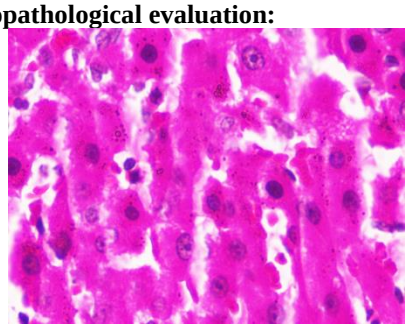

Fig 1- Control - rat

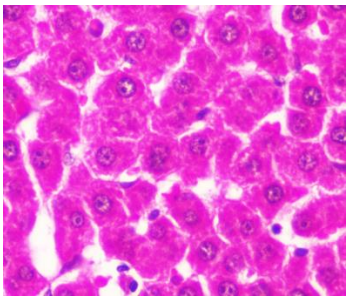

Fig 2- CCl_4 - induced rat

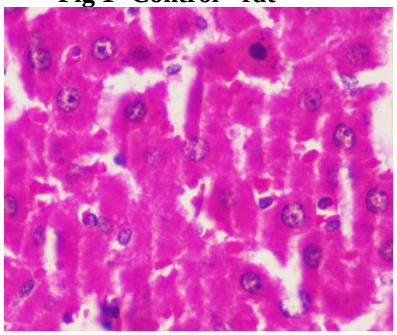

Fig 3- Standard- rat

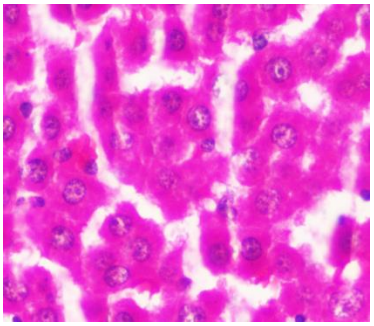

Fig 4- EA *VI* 100mg/Kg - rat

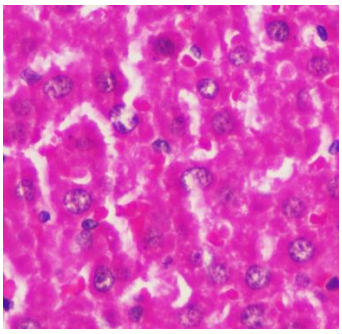

Fig 5- EA*VI* 200mg/Kg - rat

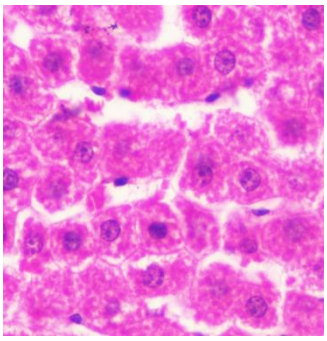

Fig 6- ME*VI* 100mg/Kg - rat

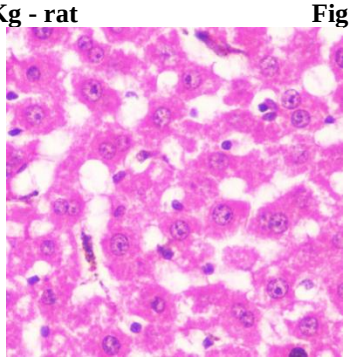

Fig 7- ME*VI* 200mg/Kg - rat

(Fig -1) Normal, (Fig -2) section of liver tissue of CCl_4 30% in olive oil (3 ml/Kg)-treated group showed massive coagulated necrosis, haemorrhage and inflammation. (Fig-3) specimen of liver tissue of CCl_4 30% in olive oil (3 ml/Kg) + Silymarin 100mg/Kg treated on the liver showed protection of normal hepatocytes. (Fig-4) Section of liver tissue treated with CCl_4 30% in olive oil (3 ml/Kg) + Ethyl acetate extract of *VI* 100mg/Kg showed tissue necrosis and inflammation. (Fig-5) Section of liver tissue treated with CCl_4 30% in olive oil (3 ml/Kg) + Ethyl acetate extract of *VI* 200mg/Kg showed tissue showed mild and moderate inflammation. (Fig-6) Section of liver tissue treated CCl_4 30% in olive oil (3 ml/Kg) + Methanol extract of *VI*100mg/Kg, liver tissue showed necrosis and inflammation. (Fig-7) section of liver tissue treated with CCl_4 30% in olive oil (3 ml/Kg) + Methanol extract of *VI* 200mg/Kg showed normal histology with mild inflammation (eosin and haematoxylin staining at 45x magnification).

DISCUSSION:

The existing study exposed the hepatoprotective activity of *V. indica* in CCl_4-hepatic toxicity in rat models. The CCl_4-induced liver toxicity includes the generation and action of free radicals on liver cells, leading to cell death is called as necrosis formation in liver cells. These cellular radicals are binds to proteins and DNA, and to cellular proteins to form a protein adducts (is a complex that forms when a chemical components binds to a genetic molecule, such as DNA or protein) [7]. This protein adducts cause the dysfunction and death of liver cells, leading to hepatic necrosis [8]. Thus, it is possible to undertake that free radicals generation and oxidative processes play a major role in hepatotoxicity in rats.

Phytochemical study of *V. indica* revealed that presence of a total phenolic content which have an effect of antioxidant and anti- inflammatory activity [9]. Higher total phenolic content has been known to contribute to the free radical scavenging activity of plant extracts [10], while antioxidant activity has also been related to the hepatoprotective effect of much kind of plant extracts.[11] . These results are confirmed with outcomes on the capability of *V. indica* to employ a hepatoprotective activity in rats. Likewise, *V. indica* has also been reported to contain flavonoid, saponins and tannins. These plant compounds have been stated to employ the hepatoprotective activity in rats [12], [13] and thus *V. indica* extracts are proposed as hepatoprotective activity in rats

CONCLUSION:

The present study validated that the *V. indica* extract possesses a hepatoprotective activity against CCl_4-chemical induced liver toxicity in rat models, which needs additional extensive studies for confirmation.

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